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STUDIES OF THERMAL INJURY
III. THE PATHOLOGY AND PATHOGENESIS OF CUTANEOUS BURNS AN EXPERIMENTAL STUDY *

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In Study II of this series,[1] measurements of the reciprocal relationships of time and surface temperature with respect to the capacity of thermal exposures to destroy the epidermis of man and pig were reported. This report concerns the pathogenesis and pathologic characteristics of cutaneous burns in relation to the duration and intensity of thermal exposure and to their susceptibility to organization, repair, and healing.

SOURCE OF MATERIAL

The material used for pathologic study was derived from several sources as indicated in Table I. Many of the hot water burns of both human and porcine skin were made in Study II of this series to establish the reciprocal relationship of time, temperature, and epithelial destruction. The method of their production has been fully described. Since the majority of the lesions described in Study II were not excised until they had been under clinical observation for days or weeks, many duplicate exposures were made and excised in order to observe the sequence of microscopic changes that took place between the incurrence of various types of injury and their repair. To acquire this material, approximately 60 additional hot water exposures of pigs' skin were made and examined microscopically after recovery periods ranging from a few seconds to several weeks. Additional porcine material was derived from a series of burns made by exposure to hot air at temperatures varying between 80° and 900° C.[2]

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There were two series of human burns, one comprised of 33 experimentally produced lesions which were studied clinically but were not excised for microscopic examination, and the other of skin specimens obtained at post-mortem examination of victims of accidental conflagrations.

Sections of tissue for microscopic examination were cut from specimens that had been fixed in Zenker-formol or 4 per cent formaldehyde solution. Phloxine and methylene blue stains were made routinely

TABLE I

Sources and Kinds of Material Used for Study of the Pathogenesis of Cutaneous Burns

Subject	Source of heat	Range of exposure		Range of recovery period
		Temperature	Duration	
Pig	Water	44° to 100°C.	0.5 sec. to 7.5 hrs.	1 min. to 4 wks.
Pig	Air	80° to 900°C.	0.5 sec. to 45 min.	1 min. to 3 days
Man*	Water	44° to 60°C.	3 sec. to 6 hrs.	1 min. to 4 wks.
Man	Air	?	?	Less than 1 hr.

* Lesions not excised for microscopic study.

and were augmented by sections stained with hematoxylin and eosin or by Pollak's modification of Masson's trichrome method. Many sections were stained by the Feulgen technic.

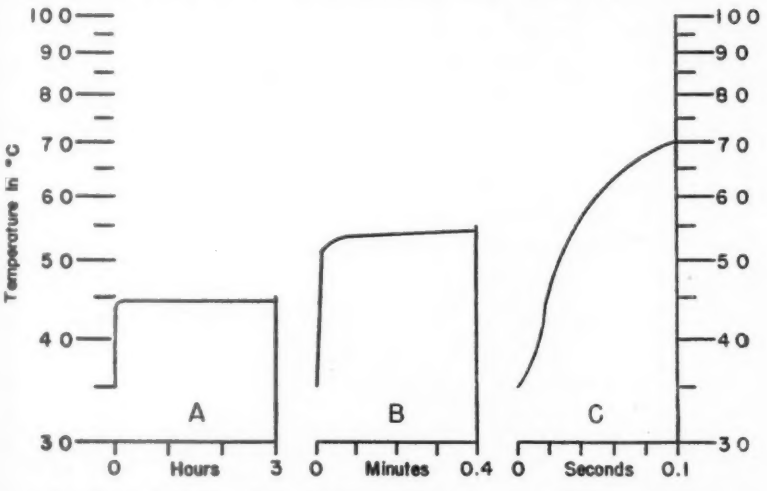
GENERAL CONSIDERATION OF THE QUANTITATIVE AND QUALITATIVE EFFECTS OF HEAT ON THE SKIN

A cutaneous injury caused by hyperthermia may be characterized quantitatively according to the depth to which the tissue has been destroyed, or qualitatively according to the nature of the changes that have occurred. The characterization in Tables II and III in Study II of hyperthermic episodes as sub-threshold, threshold, and supra-threshold refers to their quantitative capacities for injury production, the determining factor being whether the exposure in question is insufficient, just sufficient, or more than sufficient to cause transepidermal necrosis.

Thus, any exposure that failed to cause complete destruction of the epidermis was designated as sub-threshold and any reaction short of transepidermal necrosis was one of the first degree. A second degree reaction was one caused by the shortest exposure at any given temperature, or the lowest temperature at any given time that resulted in full-thickness destruction of the epidermis. Although it was not possible to destroy the entire thickness of the epidermis without some damage to the underlying connective tissue, dermal necrosis was a

relatively insignificant feature of a threshold exposure. A third degree reaction was one caused by an exposure that was supra-threshold in respect to time or temperature and was accordingly one in which a significant degree of dermal necrosis usually accompanied the destruction of the epidermis.

When account is taken of the potential variations in the intensity and duration of the different thermal exposures that are capable of producing burns of similar severity, it becomes apparent why thermal lesions may be qualitatively dissimilar even though their ultimate effect in terms of the amount of tissue destroyed is the same. This fact is more readily appreciated by reference to Text-Figures 1 and 2. The



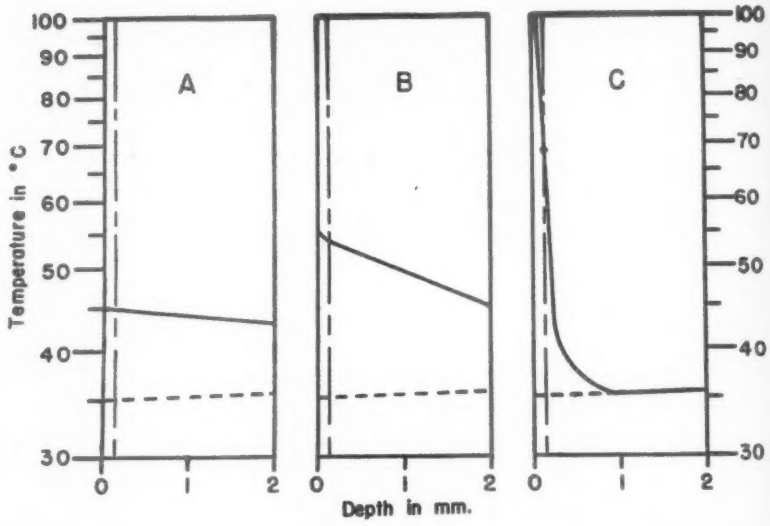
Text-Figure 1. Curves depicting the change in temperature that would occur at the interface between dermis and epidermis during surface exposures of 45° (A), 55° (B), and 100° C. (C). Each of these was a threshold exposure in that 3 hours, 0.4 minutes, and 0.1 second, respectively, are estimated to be the shortest time at the indicated temperature that would cause transepidermal necrosis. (Curves derived from data reported by Henriques and Moritz.[3])

critical temperature, so far as the ultimate fate of the epidermis is concerned, is that attained at the interface between epidermis and dermis rather than that of the surface. In Text-Figure 1 are shown the estimated changes * in temperature that would occur at the basal cell level during the course of thermal exposures at three different surface temperatures if each was terminated at a time calculated to be just adequate to destroy the epidermis. In Text-Figure 2 are shown the temperatures that would prevail at different depths below the sur-

* Calculations based on data presented in Study I of this series.[3]

face of each at the moment that the duration of the exposure was just sufficient to cause irreversible injury of the entire thickness of the epidermis.

In each instance, the effects would be quantitatively similar, in that irreversible cellular injury would extend to, but not far beyond, the basal cell layer. That qualitative differences in the resulting reactions might exist despite their quantitative similarity can be inferred from the fact that in the exposure shown in Text-Figure 1-A, the epidermis



Text-Figure 2. Curves depicting the temperature gradient through the skin that would exist at the conclusion of three types of thermal exposure at the termination of an exposure just sufficient to cause epidermal necrosis. The solid line traversing each chart from left to right depicts the temperature gradient from the surface to a depth of 2 mm. (dermis-fat interface). The transverse broken line depicts the pre-exposure gradient through the same thickness of skin. The vertical broken line indicates the approximate depth (circa 100 μ) of the dermis-epidermis interface. In A, the surface of the skin had been maintained at 45°C. for 3 hours. At the end of this time the temperature at a depth of 2 mm. had been raised from 36° to 44° C. In B, the surface of the skin had been maintained at a temperature of 55°C. for 0.4 minutes. At the end of this time the temperature at a depth of 2 mm. had been raised from 36° to 47°C. In C, the surface of the skin had been maintained at 100°C. for 0.1 second. At the end of this time there had been no change in the temperature at a depth of 2 mm. (Curves derived from data reported by Henriques and Moritz.[8])

was destroyed by a 3-hour episode of hyperthermia, the intensity of which at no time rose above 44.8° C. at the basal cell level. Approximately the same amount of irreversible change would be sustained as the result of the exposure depicted in 1-C. In the latter

instance, the epidermis would be destroyed in approximately 0.1 second by an episode of hyperthermia in which the temperature at the basal cell level rose sharply and briefly to 70° C. The exposure depicted in 1-B falls about midway between these extremes. Although the total amount of irreversible injury is about the same in each, it is to be expected that the three lesions produced by these exposures would differ qualitatively.

An additional reason for the occurrence of qualitative differences in quantitatively similar reactions to thermal exposures of different intensity is shown in Text-Figure 2, in which are depicted the calculated transcutaneous thermal gradients to a depth of 2 mm. that would exist at the moment of completion of the same three episodes of hyperthermia that are illustrated in Text-Figure 1. In each instance, irreversible thermal injury would extend to, but not appreciably beyond, the basal cell layer. In the exposure depicted in 2-A, the temperature of the dermis to a depth of about 2 mm. would be elevated above the normal level for at least 2 hours. In the exposure depicted in 2-C, the transcutaneous thermal gradient was so steep that the resulting temperature changes in the dermis were exceedingly brief and superficial. It is apparent why the epithelial cells would be destroyed in 2-C with relatively little disturbance of the dermis, whereas in 2-A the same or even a lesser degree of epidermal injury would be accompanied by a severe and persistent vascular disturbance.

First Degree Reactions

Hyperemia, Edema, and Cyanosis

Dilatation of the superficial vessels sufficient to cause visible reddening of the skin usually followed, and, in the case of exposures of low intensity, characteristically preceded the occurrence of recognizable damage to the epidermis. An exception to this generalization was the absence of vascular reaction in animals suffering circulatory failure. In such there was frequently a depression of vasomotor irritability so profound that injurious episodes of hyperthermia of either high or low intensity failed to elicit vascular reactions despite the occurrence of extensive epidermal injury. Another circumstance in which thermal damage of the epidermis was sustained with little or no vascular reaction was when an exposure to intense heat was so brief that, although the surface was burned, there was little or no rise in dermal temperature.

In the case of flash (circa 0.5 second) exposures to flame temperatures it was possible to carbonize superficial shreds of the stratum corneum without causing sufficient rise in sub-surface temperature to

damage the basal layer of epithelial cells or to cause a perceptible vascular reaction. The initial transepidermal thermal gradients established by such exposures were so sharp that the presence of a thin film of moisture on the surface of the skin was sufficient to make the difference between reaction and no reaction in the case of near-threshold exposures.

Attention has already been called to the fact that the duration of an episode of hyperthermia of low intensity must be greatly prolonged if it is to produce an injury quantitatively comparable to one resulting from an exposure of high intensity. Since the dermal blood vessels are far more responsive to temperature changes than are the epithelial cells, it can be understood why severe and persistent vascular reactions were often elicited by protracted episodes of hyperthermia of low intensity that failed to harm the epidermis (Text-Fig. 1).

There was considerably greater variation among human subjects than among pigs in respect to the vascular reactions to cutaneous hyperthermia. The variability of dermal vascular reactions in human subjects was so great and the number of reactions studied in this investigation so few that little could be inferred as to the extent to which animal data can be applied to man in this respect. The impression was gained that the thermal stimulus necessary to cause visible erythema in most human subjects was substantially lower than that required to elicit erythema in the pig. In man the change in skin color was usually more intense and of longer duration than after an identical exposure in the pig.

That an active circulation of blood was maintained through the dilated capillaries of an evanescently erythematous skin was indicated in part by the pink or red color of the surface and in part by the fact that the surface temperature * during such a reaction was characteristically between 0.5° and 1.5° C. higher than that of the adjacent skin.

An evanescent erythematous reaction to heat could not, as a rule, be recognized in sections prepared for microscopic examination. Vessels, the seat of physiologic dilatation, usually contracted during or immediately after excision, and it was difficult or impossible to distinguish a sample of physiologically hyperemic skin from one that was normal or even ischemic.

When cutaneous hyperthermia was prolonged to between 40 and 60 per cent of the minimum time required for the production of transepidermal necrosis in either man or pig, it characteristically resulted in a severe and pathologic vascular reaction with edema and cyanosis,

* Determined by means of the fine wire thermocouple described in Study VIII.[4]

and one which persisted for days rather than minutes or hours. That the flow of blood through the dilated capillaries was slowed was indicated by the blue or purple color of the surface in contrast to the pink or red color caused by the more evanescent active hyperemia. The surface temperature of such a lesion during the first few hours was frequently found to be from 0.5° to 2° C. below that of the adjacent normal skin. That the reaction was pathologic rather than physiologic was indicated also by the fact that in both man and pig it was almost invariably accompanied by cutaneous edema. Within the first hour after the onset of a vascular injury of this grade, the water content of the dermis was increased by as much as 100 per cent.

Microscopic examination of reactions of this type at varying periods after exposure in the pig confirmed the clinical observation in both pig and man that thermal exposures of relatively low intensity may result in severe and protracted disturbances of the dermal blood vessels without causing irreversible damage to the overlying epithelial cells. The capillary loops of the dermal papillae became dilated, elongated, and filled with closely packed masses of erythrocytes. Separation of collagen fibers by edema fluid was obvious and perivascular mantles of extravasated erythrocytes were often seen. The escape and extravascular deterioration of erythrocytes were often sufficient to result in brown discoloration of the target area for as long as 1 week, due to the formation of hemosiderin. Extravascular fibrin was not encountered nor did the collagen fibrils appear to be swollen. Between 12 and 24 hours after such an injury was sustained, occasional polymorphonuclear leukocytes were found in the edema fluid. Neither thrombosis nor visible alteration in the vascular endothelium was seen despite the fact that superficial vessels were filled by static, sausage-like agglomerates of red blood cells.

Reversible Impairment of Epidermal Anchorage

During most, and possibly all, injurious episodes of cutaneous hyperthermia in which the temperature of the epidermis was maintained for a sufficient time at 49° C. or higher, there was a brief interval subjacent to the threshold for transepidermal necrosis during which there was reversible impairment of the adhesion of epidermis to dermis.

The attainment of this degree of injury was recognized by the ease with which the epidermis could be dislodged by friction. If the exposure was discontinued before further injury was sustained and if the loosened epidermis was not dislodged by trauma or vesication, the change was potentially reversible in the case of the pig. After

12 or 18 hours the original firm anchorage of the epidermis was usually regained. Unless the exposure had been excessive, such injuries subsided without evidence of cell death.

When skin altered in this manner was protected against mechanical artifact, there was no microscopic evidence in either the epithelium or the underlying dermis by which impairment of the epidermal anchorage could be recognized. However, when sufficient friction was applied to the temporarily insecure porcine epidermis to cause its detachment, microscopic examination revealed a fringe of uprooted or fractured tonofibrils protruding from the lower ends of the detached basal epithelial cells. The protruding fibrils appeared to have been pulled out of their anchorage in the superficial dermal feltwork of collagen fibers. It was not determined whether the essential change responsible for such epidermal instability was a deterioration of the extracellular extensions of the tonofibrils, which predisposed them to rupture, or a softening of the dermal collagen in which they were embedded. The latter was considered the more plausible explanation of the phenomenon. In man it is doubtful that the tonofibrils of the epidermal cells have much if anything to do with the attachment of epidermis to dermis. In human skin, the epidermis appeared to be cemented to, rather than rooted in, the dermal collagen.

It has already been indicated that when porcine skin sustained this type of cutaneous burn, recovery sometimes took place within 24 hours without death of cells, providing the damaged area was protected against mechanical disturbance during that period when its anchorage to the dermis was insecure.

Too few appropriate specimens of human burns were available for microscopic examination to permit conclusions regarding the threshold at which, or the frequency with which, this particular type of first degree thermal injury occurs in man. The opinion was gained from clinical observations of human burns that thermal exposures insufficient to cause primary epidermal necrosis may result in a temporary impairment in the adhesion between epidermis and dermis. If such a temporarily insecure layer of epidermis is detached by friction or vesication, it will undoubtedly die if it remains dislodged. It is entirely possible that the phenomenon of vesication results, in some instances, in secondary destruction of human epithelial cells that would otherwise survive. If this be true, and if the thermal exposure has been insufficient to cause primary transepidermal necrosis, the immediate institution of pressure to prevent epidermal displacement by vesication should predispose to early and uncomplicated healing of what might otherwise become an open lesion.

Irreversible Thermal Injury of Epidermal Cells

Material for microscopic study was available from almost every conceivable kind, grade, and stage of thermal injury of the skin of the pig. Although a wide range of experimental thermal injuries of human skin was studied clinically, most of the burns that were available for microscopic examination were obtained from autopsies. Thus, there was no direct information regarding the intensity or duration of the thermal exposures that were responsible for the burns of human skin that were studied microscopically. The impression was gained however, that apart from the phenomenon of vesication, the cytologic changes induced by heat in the epidermis of man were similar, if not identical, to those observed in the pig (Figs. 1 to 6). Attention has already been directed to the fact that the time-temperature threshold for the destruction of epidermis is almost identical in human and porcine skin.

The first microscopic evidence of irreversible thermal injury of the epidermis consisted of what appeared to be a change in the distribution of water and solids within the nuclei of the cells of the intermediate zone. As the nuclei swelled, their chromatin granules coalesced to form compact crescentic masses immediately beneath and attached to one side of the nuclear membranes. When the swollen nucleus ruptured, the peripherally distributed chromatin contracted to form a dense and irregularly shaped central mass which remained separated from the surrounding cytoplasm by clear fluid. This fluid, whether extruded into the nuclear lacuna or contained within the distended nuclear membrane, was faintly basophilic and sometimes contained a few, fine, Feulgen-positive fragments of chromatin.

Pyknosis of nuclei was by no means pathognomonic of thermal injury. Spontaneous nuclear pyknosis was sometimes seen in the upper zone of unheated epidermis and was caused by injuries other than heat.

In the case of sub-threshold exposures sufficient to injure the upper layers of epidermal cells, but insufficient to cause transepidermal necrosis, the above-described types of nuclear change were frequently focal and difficult to distinguish from qualitatively similar changes in control material. Even though it could be plausibly assumed that all of the cells at a given level were exposed to the same degree of hyperthermia, it was not uncommon to find groups of cells with normal appearing nuclei interspersed among those with nuclei that showed advanced degenerative change (Fig. 2). The reason for this apparent difference in the susceptibility of cells in the same layer to heat was not apparent.

When the thermal exposure was of sufficient intensity or duration to cause irreversible cellular injury, nuclear changes of the kinds de-

scribed in the foregoing paragraphs were usually apparent immediately after the conclusion of the episode of hyperthermia. This was not invariably the case, however, and after certain exposures at relatively low temperature (under 47° C.) a post-exposure interval of between 6 and 12 hours was sometimes required for the development of recognizable nuclear alterations. Moreover, when the exposure was of sufficient severity to cause pseudovesication in the pig or true vesication in man, many of the nuclei which were apparently undamaged at the conclusion of the exposure disintegrated during the next 24 hours.

When the episode of hyperthermia was such as to cause visible alterations in nuclear structure, there was inhibition of mitotic division throughout the entire area of exposure for many hours. There was no evidence derived from the microscopic study of sub-threshold exposures to indicate that hyperthermia predisposed to acceleration of mitotic activity. The impression was gained that nuclear swelling with coalescence of chromatin granules constituted evidence of an irreversible cellular change and invariably led to premature death and desquamation of the altered cells.

In the pig, the irreversibly damaged epidermal cells were gradually desquamated over a period of 1 week to 10 days in the form of thin brown scales.

Alterations in the appearance of nuclei in the upper and intermediate layers of epithelium were thought to provide the earliest morphologic evidence of primary thermal injury of the epidermis and were frequently encountered without perceptible damage to the cells of the basal layer. Characteristically, the earliest change in the basal cell layer caused by hyperthermia was cytoplasmic rather than nuclear. The injured basal cells swelled and their cytoplasm became vacuolated and increasingly acidophilic. The vacuolization appeared to be due in part to imbibition of fluid and in part to redistribution of water and solids within the cells.

The fluid contained within the cytoplasmic vacuoles was clear, nonsudanophilic, and sometimes contained a delicate mesh of granular amphophilic débris. With severe injury there was widespread rupture and disintegration of the lower ends of the basal cells.

Second Degree Reactions

Transepidermal Necrosis

The time-temperature characteristics of exposures just sufficient to cause transepidermal necrosis in both man and pig are indicated in Text-Figure 4 of Study II. In man, it could usually be determined whether or not a thermal exposure had destroyed the epidermis by

the occurrence or nonoccurrence of vesication within the first 24 hours. To recognize with certainty during the first day or two after a near-threshold exposure whether or not porcine epidermis had been destroyed it was necessary to excise the skin and examine it microscopically. When the area of injury was 7 mm. in diameter and when the duration and intensity of the exposure was at or not far above the threshold required for transepidermal necrosis, the time usually required for complete healing was between 5 and 10 days in the pig and between 1 and 2 weeks in man.

In the pig, microscopic evidence that an exposure had been sufficient to cause transepidermal necrosis was provided by the changes that had occurred at the basal cell level. With the disintegration of the cytoplasm of the proximal or lower ends of the injured basal cells, there was at first, focal, and later, extensive, spontaneous detachment of the epidermis from the dermis. In the pig, the amount of fluid that collected beneath the loosened epidermis was never sufficient to produce true vesication.

With still more severe hyperthermia, the cytoplasmic disintegration of the basal cells was followed by nuclear changes similar to those seen in the more superficially located cells. When the epidermal detachment was incomplete, stretching and attenuation of the remaining bridging cells and their nuclei were often seen. Such attenuated masses of chromatin were often stretched to two or three times the original length of the entire cell.

In the event that the surface temperature of the epidermis was brought rapidly to a level of 55° C. or higher, and maintained at such a level long enough to produce a third degree burn, transepidermal coagulation usually masked any nuclear swelling that may have occurred. In such an event, neither the cytoplasm nor the nuclei of the epithelial cells appeared swollen (Fig. 7). On microscopic examination, both appeared shrunken, the former being intensely acidophilic and the latter small and homogenously basophilic.

Vesication

Attention has already been called to the fact that a common effect of heat on the skin of both man and pig is impairment of the attachment of the epidermis (Figs. 5 and 6) to the dermis, and the opinion was expressed that this may have been due either to a change in the physical state of the superficial dermal collagen or to disruption of the basal layer of epithelial cells. A common collateral effect of cutaneous hyperthermia, and one that was apparently essential to true vesication, was an outpouring of fluid from the dermal capillaries.

When a thermal exposure of human skin was sufficient to impair the attachment of the epidermis, the amount of edema fluid that collected between it and the dermis was usually sufficient to elevate and stretch the entire layer of dead, dying, and living cells to form a vesicle. Although vesication of human skin was usually an almost immediate response to a thermal exposure of sufficient severity to cause primary epidermal injury, there were several circumstances in which it was either delayed or inhibited.

Delayed vesication was most frequently seen after exposures of long duration at low temperatures or after flash exposures at high temperatures. In both circumstances it seemed likely that the delay was due to the fact that the vascular damage was relatively mild, and that hours rather than minutes were required for enough fluid to collect beneath the damaged epidermis to form a vesicle. Also vesication was delayed or prevented when the injury was so overwhelming that the dermis and its superficial capillaries were almost immediately coagulated. With such thermal injury, the level at which edema developed was too deep to result in vesication (Fig. 8). Thus, in man, the nonoccurrence of vesication after a thermal exposure sufficient to cause severe injury of the epidermis may mean that the dermal hyperthermia was either inadequate to result in edema or that it was so overwhelming that the superficial capillaries were almost immediately occluded.

In no instance was true vesication of porcine skin observed. This was true despite the fact that many of the injuries met a prerequisite to vesicle formation, namely, sufficient impairment of the adhesion between epidermis and dermis to permit easy mechanical detachment of the former (Fig. 5). Failure of the pig to vesicate appeared to be due to the fact that the amount of edema fluid that penetrated the surface of the dermis was never sufficient to elevate the epidermis. In the absence of evidence to the contrary, a tenable explanation for nonvesication in the pig was that an episode of hyperthermia that was sufficient to impair the attachment of the epidermis to the dermis characteristically altered either the superficial feltwork of dermal collagen fibers or the walls of the capillaries contained by it, in such a way that they became virtually impermeable to edema fluid.

The nature, or for that matter, the existence, of this theoretical alteration in the permeability of the collagen or the capillary walls was not disclosed by microscopic examination. When the severity of an exposure was considerably in excess of that required to destroy the epidermis, swelling of the superficial dermal collagen and occlusion of its capillaries could be recognized. There was a wide range of exposures between the threshold for epidermal necrosis and that for recognizable

swelling of collagen or occlusion of capillaries in which the microscopic examination of the pig's skin disclosed no explanation for failure of porcine skin to vesicate.

Third Degree Reactions

The more a thermal exposure exceeded the threshold required for destruction of the epidermis, either in respect to temperature or time, the deeper the injury and the longer the recovery period necessary for repair and regeneration. In both pig and man, several weeks represented the minimum healing time if a significant degree of dermal injury had been sustained.

Further Changes in the Epidermis

In the case of the pig, prolonged exposure at a relatively low surface temperature (under 55° C.) caused relatively little additional change in the microscopic appearance of the epidermis. In the higher range of surface temperatures, a significant prolongation of the rate of duration of exposure beyond the time necessary to destroy the epidermis modified the quality of the superficial changes both in human and porcine skin. In man, vesication was permanently inhibited and in both man and pig the loosened epidermis became reattached to the damaged dermis (Figs. 7 and 8). Early shrinkage of both the cytoplasm and the nuclei of the damaged cells occurred before there was opportunity for the development of the retrogressive changes observed in first and second degree reactions. The higher the temperature, the shorter the time required to cause transepidermal coagulation. With exposures to superheated air, desiccation was superimposed on the effects of heat, and soon after the temperature rose above 300° C., carbonization of the dry tissue began to take place.

Red and Pale Burns

The surface color of third degree burns ranged from pale gray through red, purple, and brown to black, depending on certain attributes of the exposures responsible for their production.

A black or carbonized surface resulted from exposures at temperatures in excess of 300° C. (Fig. 9). The precise temperature at which carbonization began was not determined.

A red, purple, or brown surface, due to the presence of blood in the superficial layer of the skin, resulted from exposures in which the dermal temperature was raised slowly enough to permit advanced engorgement of the superficial capillary plexus before the occurrence of coagulation.

A gray or ischemic surface indicated that the upper portion of the dermis had undergone thermal coagulation before the superficial capillaries had become fully engorged.

The reciprocal relationships of time and temperature as they relate to the visibility of hemoglobin beneath the surface of a third degree thermal reaction are shown in Table II of Study II. It was found that at atmospheric pressure and at surface temperatures of 65° C. or lower, burns appeared superficially hyperemic regardless of the duration of exposure. When a 70° C. surface exposure was interrupted at the end of 2 seconds, the lesion remained red, but if it were prolonged

TABLE II

Increase in Hemoglobin Iron in Skin and Subcutaneous Tissue following Thermal Injury

Condition of skin	Weight of iron per sq. cm. of sample	Estimated weight of blood per sq. cm. of sample
	(μg.)	(mg)
Normal	2.4	5.5
	4.0	9.0
Moderate hyperemia	5.6	13
	12	26
Severe hyperemia	22	50
	24	55
	16	35
	16	35
	15	34

to 3 minutes, the zone of reactive hyperemia became overlaid by so thick a layer of coagulated tissue that it was no longer visible. Above 70° C. all exposures of 1 second or longer coagulated the superficial plexus of dermal capillaries so rapidly that most or all of the blood contained in them was displaced to a level too deep to be seen from the surface.

POOLING OF BLOOD IN HYPEREMIC BURNS

A qualitative impression of the pooling of blood in the dilated cutaneous vessels after an injurious episode of hyperthermia was derived from the photomicrographs shown in Text-Figure 2 of Study II. In order to learn something of the actual amount of blood that was present in such lesions, samples of both normal and hyperemic skin were excised for chemical examination. Samples of skin and subcutaneous tissue having an area of 25 sq. cm. and extending to the deep fascia were taken from the lateral thoracic area of each of 9 pigs and their iron content was determined.[5] Two of the samples represented normal skin and the other 7 were from areas of hyperemic burning.

It is apparent from the results of the experiments shown in Table II that a relatively large proportion of the total circulating blood of an animal may be pooled in the skin and subcutaneous tissue as a result of thermal injury. Calculations based on the amount of recoverable iron per unit of surface area of burned skin in relation to the body weight indicated that as much as 30 per cent of the erythrocytes of an animal suffering from generalized cutaneous hyperemia could be accounted for in the skin and subcutaneous fat.

THE EFFECT OF COMPRESSIVE HYPERTHERMIA ON THE COLOR OF A BURN

In Study II, attention has been called to the fact that pressure on the skin surface during exposure to heat does not increase the vulnerability of the epidermis to thermal injury except as it may improve the contact between a hot solid and the skin surface. It was found, however, that compression of the skin was capable of modifying the superficial color of the burn even though there was no increase in the severity of the lesion. To determine the circumstances in which compression of the skin during an episode of hyperthermia may modify the color of the burn, the experiments on pigs summarized in Table III

TABLE III

The Effect of Pressure upon the External Appearance of Burns

Temperature of surface	Duration of hyperthermia	Pressure on skin	External appearance of burn 24 hours after exposure	
			Ischemic	Hyperemic
(°C.)	(*seconds*)	(*mm. Hg*)		
70	5	0		+
	5	120	+	
65	30	0		+
	30	120		+
	60	120	+	
	1200	0		+
60	60	0		+
	60	120		+
	120	120	+	
	300	0		+
55	1800	0		+
	1800	120		+

were undertaken. In some, hot water was applied at atmospheric pressure, and in others it was applied with a compressive force of 120 mm. of Hg.

The results of these exposures indicated that the color of burns resulting from surface temperatures lower than 55° C. was not affected by pressure, but that an increase in pressure during exposures at

surface temperatures of 60° C. or higher determined whether the surface of the resulting burn would be ischemic or hyperemic. Thus, an exposure at atmospheric pressure at 60° C. produced a red burn even though it was extended for as long as 5 minutes. With increase in pressure, a 2-minute exposure at the same temperature resulted in a pale burn and yet the depth to which the tissue had been destroyed in the latter was less than that to which it had been destroyed in the former. At 70° C., a 5-second exposure at atmospheric pressure resulted in a red burn, but with an additional pressure of 120 mm. of Hg the resulting burn appeared ischemic.

Microscopic examination of these lesions provided evidence that the color of a burn was not a reliable criterion by which to judge its depth. After hyperthermic episodes of comparable duration and at the same surface pressure, a red surface color usually indicated that the lesion was less severe than one having a gray surface. Without knowledge of time, temperature, or surface pressure during the period of exposure, it is not possible to estimate the relative severity of burns on a basis of surface color.

OTHER EFFECTS OF HEAT ON THE DERMIS

After edema and pericapillary extravasation of erythrocytes, the earliest recognizable extravascular alteration of the dermis was swelling of collagen fibers. This occurred first in the most superficial layer where, in the case of porcine skin, the projecting tonofibrils of the basal epithelial cells were imbedded in the collagen of the subjacent connective tissue.

As the intensity and duration of the hyperthermia increased, the corium tended to lose its fibrillar character and became a thin lamella of homogeneous acidophilic material as though the individual fibers had been converted to a gel. With increasing exposure the swelling of collagen became apparent at greater and greater depths in the underlying connective tissue (Fig. 8). Expansion of collagen bundles tended to collapse and obliterate the loose perivascular areolar tissue. Contracted blood vessels appeared thick-walled and empty. Visible edema receded in advance of this type of alteration as though the fluid were imbibed or displaced by the denatured collagen. Not until 24 or 48 hours had elapsed was it possible by microscopic examination to recognize the line of demarcation between reversible and irreversible dermal injury.

From the intact blood vessels of the deeper and relatively uninjured tissues leukocytes migrated upward through the perivascular interstices and into the zone of denatured collagen. A frontier was eventu-

ally established between the tissue capable of recovery and that destined to be sequestered in the form of a desiccated crust. The deeper the lesion the longer the time required for the stabilization of such a frontier. The transition between the obviously necrotic tissue of the upper dermis and the least disturbed tissue of the deepest portion of the zone of hyperthermia was a gradual one.

Exudation of leukocytes occurred within a few hours, and within 24 hours usually served to delineate the zone within which the plane of irreversible injury would eventually become stabilized. Within 2 or 3 days, fibroblasts and new capillaries began to push up toward the surface in the interfascicular interstices of the denatured collagen (Fig. 10). The least affected connective tissue at the base of the reaction zone recovered quickly and without apparent loss of fixed tissue cells. The fate of the more severely injured collagen varied according to the extent to which it had been denatured. Thermal denaturation of collagen at temperature levels under 55° C. (Fig. 11) did not appear to result in the kind of coagulative change that made the collagen resistant to subsequent autolysis and organization. Collagenous denaturation at temperatures over 55° C. often resulted in an irreversible type of coagulation which resisted lysis and eventually led to sequestration en masse. Thus, deep and severe burns resulting from surface exposures lower than 55° C. were likely to remain soft and red and the necrotic tissue was susceptible to organization. Deep burns resulting from higher temperatures were characteristically firm and pale and there was sequestration but not organization of the necrotic tissue. Between these two extremes the dead and damaged connective tissue was infiltrated by leukocytes and penetrated by granulation tissue, and its necrotic elements were gradually resorbed and replaced by new connective tissue.

During the time required to establish the level of irreversible injury, tentative tongue-like masses of new epithelial cells grew out from the margins of the lesion and from the viable roots of partially destroyed hair follicles as though they were seeking a sufficiently well stabilized layer of connective tissue to provide support and nutrition. Repeated crops of such new epithelial cells extended over or into the granulation tissue and failed to survive, for reasons not disclosed by microscopic examination.

Summary

The transfer of heat to the skin at a rate sufficiently great to raise the sub-surface temperature to an appreciably higher level than that which is normal for the organism leads to a series of local reactive and alterative changes, the severity of which bears a direct relationship

to the degree and duration of the temperature rise. The nature of the change that occurs at any given depth below the surface of the exposed skin is determined in part by the intensity and duration of the temperature rise at that level and in part by the nature of the affected tissue.

The mildest form of epidermal injury produced by hyperthermia is latent in the sense that it is not associated with recognizable alteration in cell structure. Such injuries are reversible and the time required for their reversal increases in direct relation to the time required for their production.

The earliest visible evidence of thermal injury of the epidermis is a redistribution of chromatin within, and swelling of, the nuclei, first in the intermediate and later in the deepest layer of epithelial cells. Further injury results in swelling and disintegration of the cytoplasm of the basal cells and pyknosis of nuclei throughout the entire thickness of the epidermis. As the result of alterations in the basal cells or in the cement substance that binds them to the dermis, hyperthermia may result first in a reversible and subsequently in an irreversible impairment of the attachment between epidermis and dermis. If the temperature rise within the epidermis does not exceed a level of approximately 55° C., further primary thermal alterations of the epithelial cells either do not occur or are so slow in development as to be of negligible significance.

When the exposure is such as to result in a progressive rise in sub-surface temperature, the next step in the succession of alterative changes is the occurrence of transepidermal coagulation. When the epidermal temperature is raised rapidly to a level higher than 55° C., coagulative changes may mask both nuclear swelling and cytoplasmic disintegration. Further increases in the temperature of the epidermis result in progressive desiccation and eventually in carbonization.

The earliest visible alterative change in the dermis in response to hyperthermia is constriction of the superficial blood vessels. Ordinarily, this is followed almost immediately by vasodilatation. When the rise in dermal temperature is sufficiently rapid and high, the superficial vessels become permanently fixed in their initial reactive state of constriction and vasodilatation occurs only at deeper levels where the temperature rise has been less extreme.

Thermal vasodilatation characteristically leads to increased vascular permeability and edema formation. The escape of edema fluid at any particular sub-surface level requires, first, that the vascular injury at that level has been sufficiently severe to result in increased mural

permeability and, second, that it has not resulted in cessation of blood flow through the damaged vessels.

Although thermal exposures of appropriate intensity and duration impair the attachment of epidermis to dermis and increase the permeability of the superficial capillary plexus of the dermis in both pig and man, it is only in the latter that a sufficient amount of edema fluid collects beneath the epidermis to cause true vesication.

Absence of vesication in human skin that has been exposed to heat may indicate that the rise in dermal temperature was insufficient to cause increased vascular permeability, that insufficient time has elapsed for the collection of enough fluid beneath the epidermis to cause appreciable elevation, that the hyperthermia was so extreme as to have caused cessation in blood flow through the superficial capillaries, or that cutaneous ischemia and loss of vasomotor irritability were antecedent to the exposure to excessive heat.

As in the case of the epidermis, coagulative changes occur in the dermis when its temperature is raised to, and maintained for a sufficient period at, a level higher than 55° C. Further increases in dermal temperature may result in desiccation and carbonization.

It was found that the superficial appearance of a burn is not a reliable criterion by which to judge the depth to which the tissue may have been irreversibly injured. The surface of a relatively shallow burn may show coagulation and even carbonization if the exposure has been intense and brief, whereas a thermal exposure of insufficient intensity to coagulate even the most superficial portions of the skin may, nevertheless, cause deep destruction if sufficiently prolonged.

Although the quantitative results of a short exposure of high intensity may be similar to those of a long exposure at low intensity, there are likely to be significant qualitative differences between such injuries. Hyperthermia of high intensity results in a coagulative type of necrosis in which the dead tissue does not undergo autolysis, resists organization, and is usually disposed of by sequestration. Hyperthermia of low intensity results in a noncoagulative type of necrosis in which the dead tissue undergoes autolysis and is readily susceptible to organization.

Biopsy of a burned area within 24 hours should provide useful information pertaining to the depth and nature of injury. Recognition by microscopic examination of dermal coagulation would indicate eventual sequestration of the tissue so affected. Recognition of a zone of irreversible injury would indicate the need for debridement before attempting to graft new epidermis on the surface.

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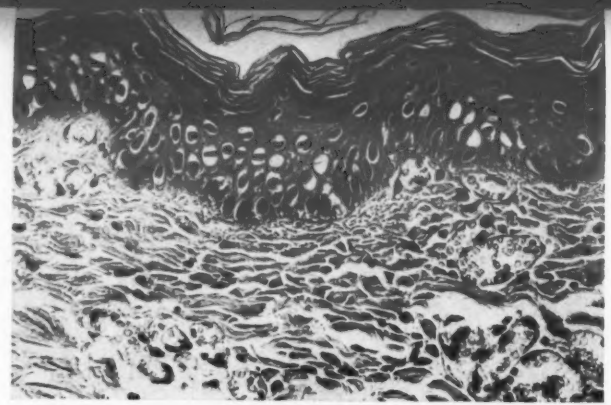
DESCRIPTION OF PLATES

PLATE 136

FIGS. 1 and 2. Photomicrographs of severe first degree burns of porcine (Fig. 1) and human (Fig. 2) skin showing the degenerative changes in epidermis. In Figure 1 there is generalized pyknosis of nuclei, and none of the epidermal cells included in the picture would have recovered. In Figure 2 the changes are focal rather than general and most of the altered nuclei are swollen and show peripheral displacement of the chromatin. This type of nuclear change precedes that shown in Figure 1. Both specimens were excised within 1 hour after the injury was sustained. In both instances the epidermal attachment to the dermis was insecure and the lesion shown in Figure 2 probably would have gone on to vesication in the normal course of events. × 400.

FIGS. 3 and 4. Photomicrographs of early second degree burns of porcine (Fig. 3) and human (Fig. 4) skin showing early spontaneous detachment of the epidermis from the dermis. Vacuolar cytoplasmic disintegration of the basal cell layer has been added to nuclear changes similar to those shown in Figures 1 and 2. In Figure 3 the tonofibrils that were uprooted from their anchorage in the dermis can be seen projecting from the detached basal cells. × 400.



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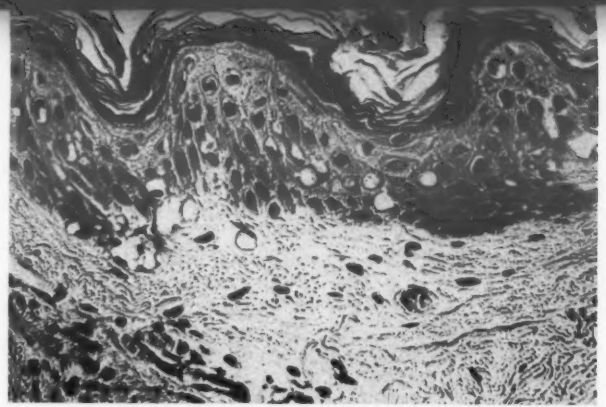

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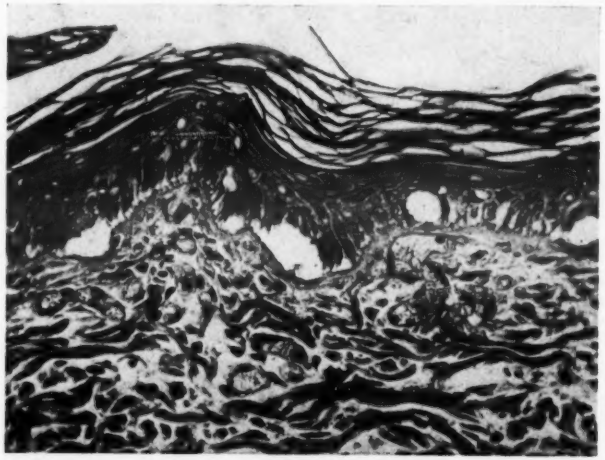

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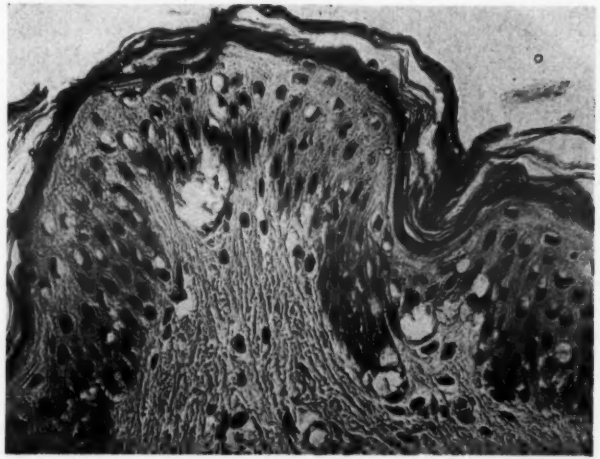

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Thermal Injury

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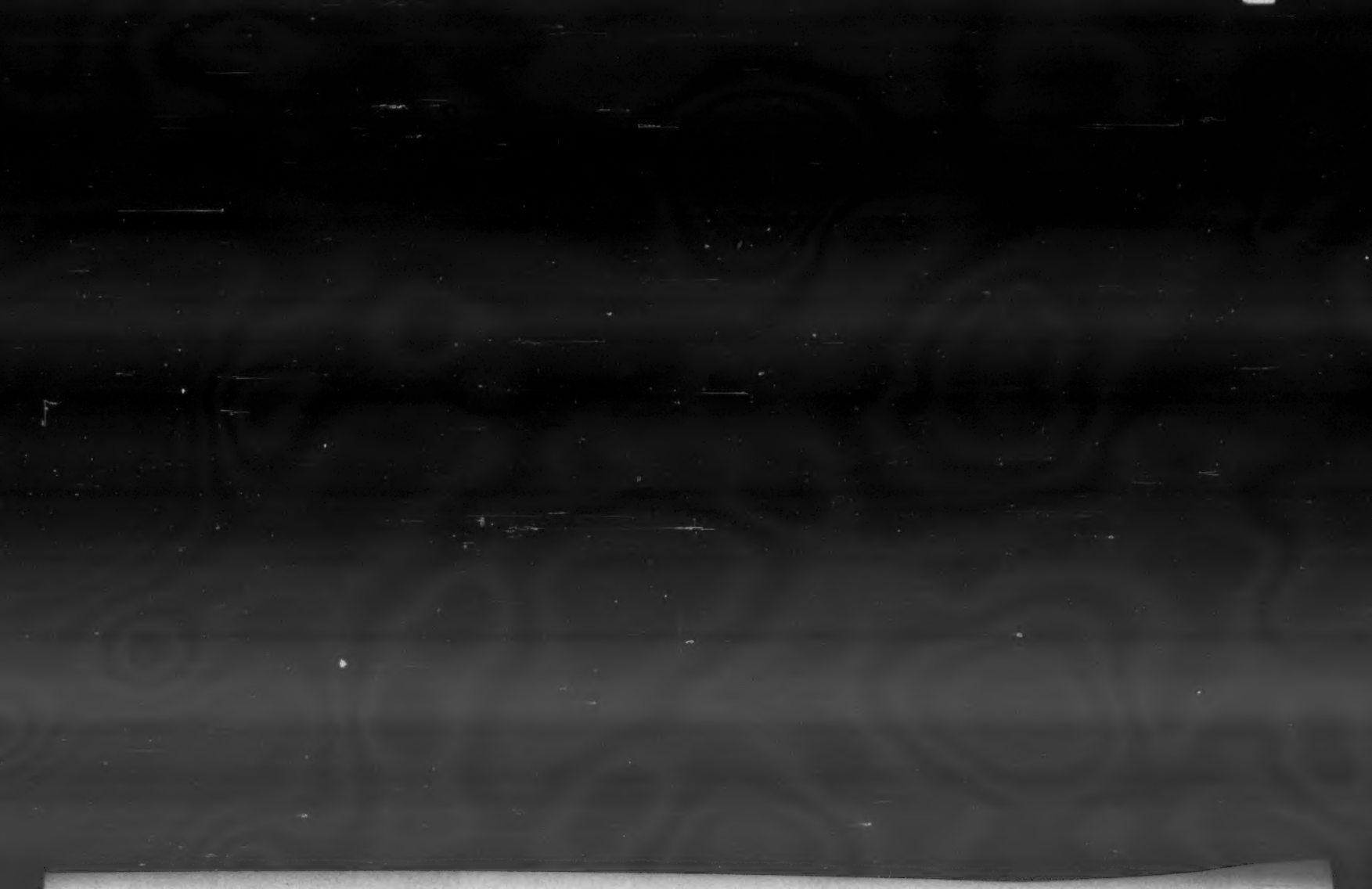
FIGS. 5 and 6. Photomicrographs of a pseudovesicle of porcine skin (Fig. 5) and an early true vesicle of human skin (Fig. 6). In each, transepidermal necrosis is complete. In the case of porcine skin the detached epidermis would have remained *in situ* as a flaccid membrane. In the case of human skin the detached epidermis would have been elevated by the collection of edema fluid between it and the dermis. × 400.

FIG. 7. Third degree thermal injury of porcine skin showing coagulation of epidermis and dermis 24 hours after injury. Burn produced by maintaining surface of skin at 65° C. for 2 minutes. The bundles of denatured dermal collagen appeared swollen and homogeneous, and had become increasingly basophilic. Thermal reactions of this type were encountered only when the surface temperature had been brought to, and maintained at, a level of 55° C. or higher. Coagulative changes of this type render the necrotic tissue resistant to lysis and organization. × 400.



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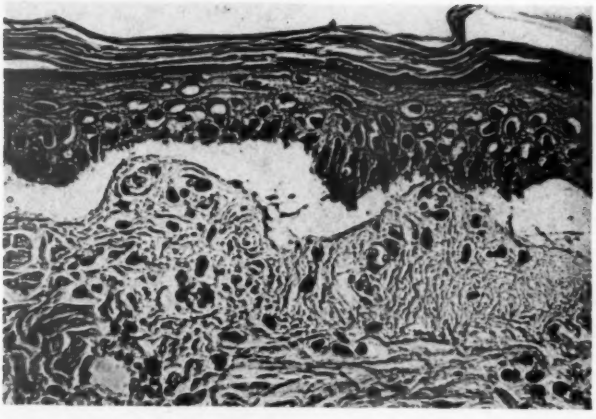

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PLATE 138

FIG. 8. Transcutaneous coagulation resulting in a deep ischemic lesion, caused by a 5-minute exposure to circumambient (air) temperature of 200° C. × 85.

FIG. 9. Carbonization of surface of skin with desiccation and intense basophilia of coagulated dermis, caused by an exposure of 2.5 minutes to a circumambient (air) temperature in excess of 400° C. × 85.

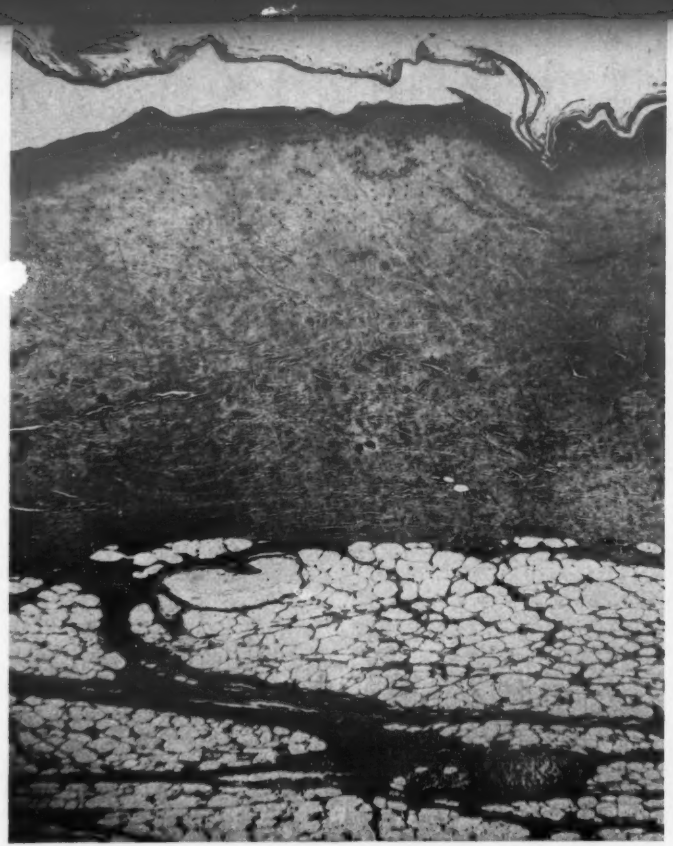

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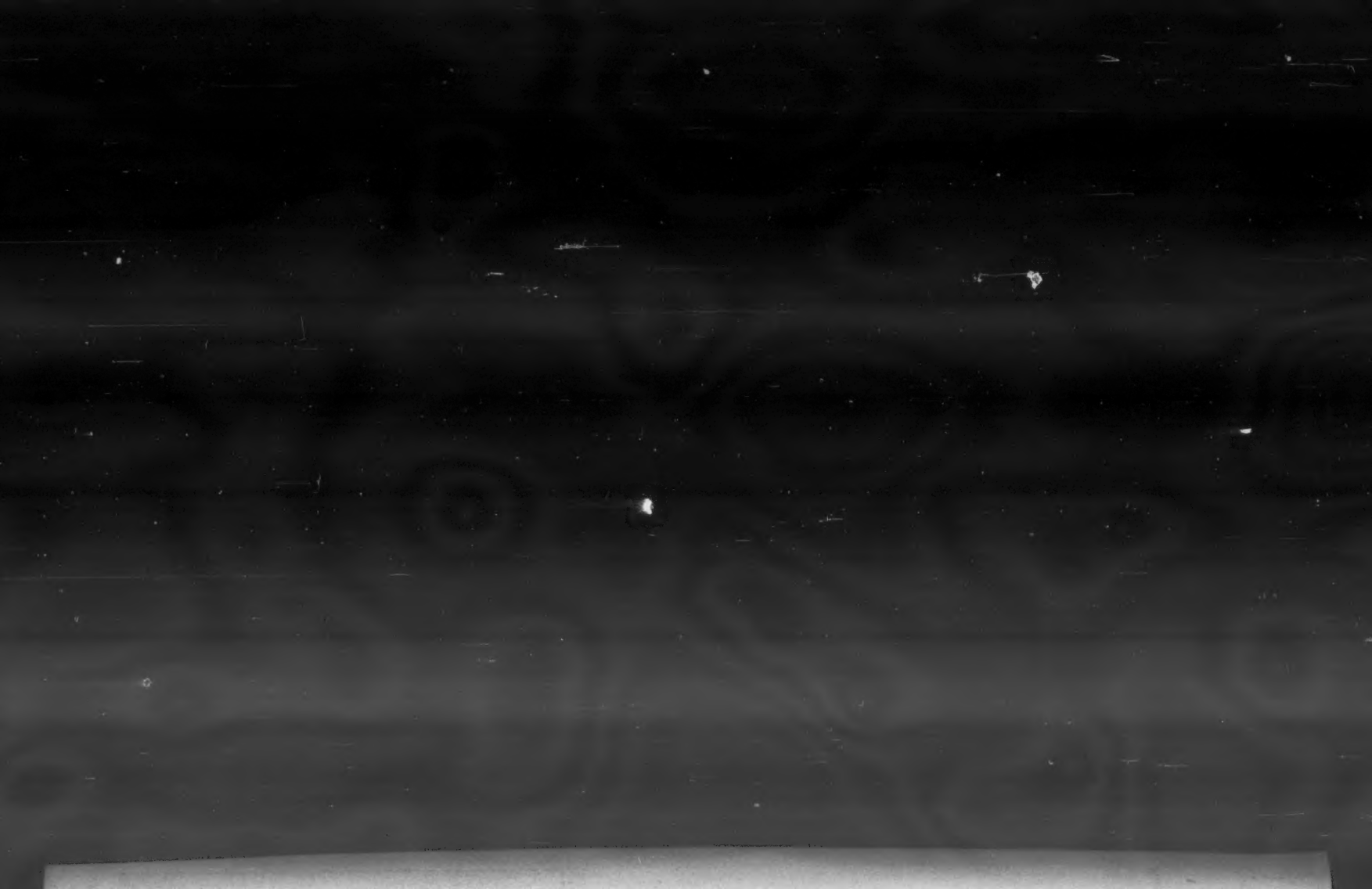
FIG. 10. Photomicrograph of a third degree thermal reaction in porcine skin 72 hours after injury. Exudative cells have migrated into the interstices between the bundles of coagulated collagen. The precise level at which this injury will be stabilized is not yet apparent. Healing will be slow because of the resistance of the denatured collagen to autolysis and organization. × 400.

FIG. 11. A 24-hour-old third degree burn of porcine skin in which the necrotic dermal tissue was not coagulated, was already densely infiltrated by exudative cells, and was readily susceptible to organization and repair. Burn produced by maintaining the surface of the skin at 53° C. for 5 minutes; for comparison with burn shown in Figure 7 in which coagulation of dermal collagen had rendered it resistant to lysis and organization. × 400.



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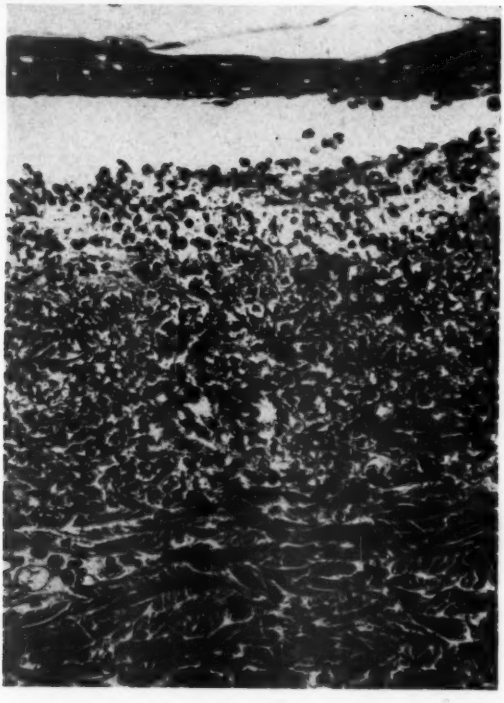

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GRANULOMA, A CHARACTERISTIC "QUALITATIVE" CHANGE IN FOCAL ANAPHYLACTIC INFLAMMATION *

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I. INTRODUCTION

Nature of the Anaphylactic Reaction. The "Quantitative" Hypothesis

The effects of Arthus' "Repeated Injections of Horse Serum into the Rabbit," [1] presented as a note to the Société de Biologie in June, 1903, became soon afterwards, under the name of "Arthus' phenomenon," the starting point of innumerable immunologic, physiologic, biochemical, and clinical researches on anaphylaxis, hypersensitiveness, and allergy. His second note, with Maurice Breton, on "Cutaneous Lesions Induced by Horse Serum Injections into Rabbits Anaphylactized by and for this Serum" [2] was presented to the same Society a few months later (November, 1903). This note, dealing with the histopathologic aspects of local shock, did not share the fate and importance of the first article, which dealt with its physiologic implication. The reason is to be found in the fact that the histopathologic picture of the focal anaphylactic response, as described therein, is not at variance with a "common" inflammatory reaction, except for some heightening of the process.

The assumption that focal anaphylaxis was pathologically similar to a common type of inflammation needed verification. Controlled researches were not performed, however, until 1914. Between 1914 and 1923, Rössle,[11,12] and, under his instigation, Fröhlich,[8] and Gerlach [4,5] attacked the problem anew. The experimental method consisted either of a reproduction of the classical Arthus' phenomenon (on rabbits, and other animals; Gerlach) or of appropriate modifications. The latter comprised (1) watching the initial stages of the anaphylactic response and their development on the transparent mesentery of a living and sensitized frog, with substitution of the shock antigen for an inflammation-inducing agent (Fröhlich); (2) studying the local lesion induced by subcutaneous injection of avian erythrocytes into specifically sensitized guinea-pigs, the local shock being elicited when the serum of the guinea-pigs reached a high titer of antiavian hemolysins (Rössle); (3) observing, through "abdominal windows," the gross peritoneal changes in specifically sensitized animals when the shock antigen is introduced into the peritoneal cavity (Rössle).

These controlled researches, which brought forth some additional

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data (presence of eosinophilia, for example) failed nevertheless to invalidate the initial findings of Arthus and Breton.[2] It is repeatedly maintained by the authors cited that the local anaphylactic reaction is not "qualitatively" different from any inflammatory response, the changes being simply and solely "quantitative." These quantitative changes concern the suddenness of the reaction; the immediately ensuing and more or less sustained blood stasis; the tremendous edema, which may or may not be hemorrhagic; the abundant eosinophilia, and the necrosis of the vessels walls. The process is heightened, accelerated, and shortened, and "stormy" in its unfolding. It fixes and limits the injurious shock antigen to its portal of entry, and therefore protects the organism as a whole, sometimes at the expense of local tissue death, through the interplay of capillary contraction, walling off by exudation, and the neutralizing and diluting effects of the edema.

Evidence Against the "Quantitative" Hypothesis

The accepted opinion, outlined above, that local anaphylaxis is only a "quantitative" variant of inflammation does not appear tenable, for the following reasons:

(a) Inflammation and anaphylaxis are two distinct phenomena. Inflammation follows a wide range of inciting agents and in final analysis is due to the injury or death of a cell or group of cells. Anaphylaxis, on the other hand, is highly specific, in that it depends upon an active or passive sensitization, a latency period, and, at least in part, on some form of antigen-antibody reaction. Anaphylaxis could be only a special case of inflammation, and as such its histologic picture should show more than "quantitative" changes.

(b) The anaphylactic response is the result of a primary antigen-antibody coupling, and occurs not in the blood stream or tissue fluids, but on or in cells which are able to remove or fix the circulating antibody. The anaphylactic state, moreover, is associated with the presence of a "fixed" antibody, and, conversely, with the absence of a freely circulating antibody. Immunologists have long since discarded the "humoral" or "anaphylatoxin" hypothesis, which supposes the formation of an antigen-antibody complex, and the adsorption by the latter of a stabilizing serum constituent with subsequent digestion of serum proteins or release of a toxic compound. Instead, immunologists now rally to the "cellular" hypothesis. It is beyond the aim of the present article to submit the experimental evidence which is so strongly in favor of the view that the anaphylactic response is cellular in nature and origin. However, we are still ignorant of whether the

phenomenon occurs at the surface or in the interior of cells, the types of cells concerned, and the histologic modalities of the phenomenon.

If, therefore, during the anaphylactic shock, the cells alone are operative, it seems fair to assume that a responsive cell may be found which will show by histologically analyzable signs its dominant rôle in the phenomenon. One may venture to say that its reactivity must be specific and "qualitatively" different from a common inflammatory response.

Rôle of the Histiocytes

Experimental evidence points to only two types of cells which seem to respond during the anaphylactic shock: the smooth muscle cell and the reticulo-endothelial cell. The reactivity of the smooth muscle cells explains, among other experimental and clinical data, the Schultz-Dale phenomenon and that of the "effector organ" (which varies from species to species, according to the richness of smooth muscle cells in these organs). However, in focal anaphylactic reactions of the Arthus type, the smooth muscle cells cannot be considered because of their scarcity in the subcutaneous tissue, where they are found only in the vessel walls. The reticulo-endothelial cells, on the other hand, are ubiquitous and can be found in the active mesenchyme of the subcutaneous tissue, not only as primitive mesenchymal cells, endothelial and perithelial cells, but also as histiocytes and monocytes.

The rôle of the histiocytes and their derivatives in anaphylaxis is still a debatable question (Jungeblut [6]), although experiments indicate a strict relationship between functional responsiveness of the reticulo-endothelial system and the anaphylactic shock. India-ink blockade attenuates and in cases inhibits shock when the specific antigen is injected. Similarly, the duplication of Arthus' initial experiment fails to induce an Arthus' phenomenon when the shock antigen is injected into a previously "blocked" area (experiment of the so-called local blockade, Klinge [7]).

Another argument in favor of the reactivity of the histiocytes is adduced by the behavior of the capillaries, which, with their potentially histiocytic endothelial and perithelial cells, are an integral part of the reticulo-endothelial system. Some of their reactions are only physiologic and not demonstrable microscopically, such as their functional insufficiency (the so-called irritability state of Doerr). In more advanced instances the damage becomes morphologically demonstrable as in the necrosis of the vessels, which is a prominent feature of the local anaphylactic reaction.

Finally, it has been shown by Rich,[8-10] both clinicopathologically

and experimentally, that in serum sickness the lesion is essentially a monocytic adventitial and perivascular infiltration.

If, then, the histiocytes and derivative cells, and the cells making up the capillaries are physiologically reactive during the anaphylactic shock, an investigation of the histologic behavior of the histiocytes as a whole, and of the endothelial and perithelial cells in particular, seems indicated.

With the above-mentioned considerations as a starting point, experiments have been performed to investigate the reactivity of the histiocytes and capillaries in focal anaphylactic response. Other experiments, concerning the behavior of the histiocytes in local anaphylactic reactions in animals blocked with trypan blue, and the histopathology of sensitized areas after injection with histamine, are in progress.

II. Experimental Data

All experiments were performed on healthy male and female guinea-pigs of 250 to 600 gm. average weight, kept on the usual diet of "kitchen greens" (cabbage, carrots, beets, etc.). Egg white was used as sensitizing and shock antigen. The sensitizing antigen was administered both intraperitoneally (for general sensitization) and subcutaneously (for focal sensitization). On occasion the sensitization of the focal area was reinforced by a subsequent subcutaneous injection given 24 hours after the first sensitizing injection. The site chosen for the focal injections was the mid-abdominal wall. Each subsequent injection, whether with the shock antigen or with the nonspecific antigen, was made in the same area as far as it was possible to do so, taking as a guide either the previous needle puncture mark or the grossly visible and palpable tumefaction.

In the identically sensitized control animals the shock antigen was replaced with nonspecific antigen (horse serum; in some experiments, human and rabbit serum). In one of the experimental groups, both the shock and nonspecific antigens were applied in dry form, as dressings, on a denuded area of the sensitized region.

The experiments are given in tabular form (Table I). The scope of each experimental group is summarized in the heading preceding its description in the text.

Most of the animals showed varying physiologic responses when the shock antigen was injected; they ranged from restlessness to polypnea, which was marked but of short duration. Only one guinea-pig showed the characteristic hair bristling over the head and neck ("lion's mane" sign), and recovered quickly.

The histologic technic was identical for all animals, and consisted

in Zenker's fixation, paraffin embedding, and staining with Mallory's phosphotungstic acid hematoxylin and with Mallory's phloxine and methylene blue. Occasionally Masson's trichrome stain and Foot's silver impregnation of reticulin fibers were used.

Group I. Inflammatory Changes Induced in Sensitized Animals by Subcutaneous Injection of Specific (or Shock) and Nonspecific (Control) Antigens

A. Shock Antigen. There were four findings of note in the animals receiving specific antigen.

(1) Presence of a cavity, heavily outlined by coarse and wavy fibrinous fibers. This cavity is believed to have contained the resorbed specific antigen.

(2) Presence of monocytes, histiocytes, and related cells in streamers or in loosely packed and ill-delimited islets. They were either scattered throughout or bordered the cavity which had been emptied of antigen.

(3) Presence of endothelial and perithelial hypertrophy and hyperplasia, of intraluminal blocking by monocytic forms, and evidence of endothelial and perithelial metamorphosis into monocytes, histiocytes, and related cells. The endothelial cells revealed their prospective potencies not only in the capillaries but also in precapillary arterioles.

(4) Presence of perivascular (Figs. 1 and 4), perineural (Fig. 3), and peri-adipose and intra-adipose granulomata (Fig. 2).

The remainder of the changes were consistent with a residual inflammation heightened by a recently superimposed acute inflammation. Edema, contiguous with the empty cavity, was tremendous and dissociated the stroma. The fragmented collagenous fibers showed swelling and occasional hyalinization, and were blurred or glassy. There was scattered erythrocytic diapedesis with no signs of a scavenger reaction. Neutrophilic polymorphonuclear leukocytes were scant, while eosinophils were numerous and lymphocytes rather rare. Venules occasionally were plugged with fibrin thrombi. Growth stimulation of fibroblasts was early.

B. Controls. In the animals which received nonspecific antigen the general picture was that of a residual inflammation with superimposed recent, mild, acute inflammatory changes and with no evidence of granulomata or of endothelial and perithelial reactivity. Coarsely granular coagula, presumably incompletely resorbed nonspecific antigen, were demonstrable. Edema was scant to moderate. Eosinophils were rare. In the scattered small areas of hemorrhages, groups of red cells were surrounded by macrophages.

TABLE I

Tabular Summary of Protocols of Experiments

	Group I		Group II	
Experiment	A	B (control)	A	B (control)
Number of guinea pigs	4	2	3	2
Day 0	1 ml. egg white intraperitoneally; 1 ml. egg white subcutaneously (sensitization)		3 ml. egg white intraperitoneally; 4 ml. egg white subcutaneously (sensitization)	
1	1 ml. egg white subcutaneously (reinforcement of local sensitization)			
10				
11			Linear incision, 1 cm. long on sensitized area. Wound dressed twice daily with:	
			Powdered egg albumin (shock antigen)	Powdered horse serum (nonspecific antigen)
12			Dressings as above	
13	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
14	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
15	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
16	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
17	*	*	*	*
20				
29				
31				
Granulomata	Present	Absent	Present	Absent

* Animals sacrificed.

Group III				Group IV			
A	B	C	D (control)	A	B	C	D
1	1	1	1	3	3	3	3
3 ml. egg white intraperitoneally; 4 ml. egg white subcutaneously (sensitization)				2 ml. egg white intraperitoneally; 3 ml. egg white subcutaneously (sensitization)			
4 ml. egg white subcutaneously (reinforcement of local sensitization)				3 ml. egg white subcutaneously (reinforcement of local sensitization)			
				4 ml. egg white subcutaneously (shock antigen)	3 ml. horse serum subcutaneously (nonspecific antigen) (re-sensitizer)	3 ml. human serum subcutaneously (nonspecific antigen)	
4 ml. egg white (shock antigen) subcutaneously—diluted			4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10					
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
*	*	*	*				
				4 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen becomes shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	
				4 ml. egg white subcutaneously	3.5 ml. horse serum subcutaneously	3 ml. rabbit serum subcutaneously (nonspecific antigen)	3 ml. egg white subcutaneously (shock antigen)
				*	*	*	*
Present	Present	Present	Present	Present	Present	Absent	Present

* Animals sacrificed.

Group II. Wound Healing Type of Inflammation. Topical Application of Shock and Nonspecific Antigens, in Dried Form

A. Shock Antigen. In the animals given specific antigen the denuded area was covered by fibrin-entangled ghosts of polymorphonuclear leukocytes, which rested upon an irregularly patterned network of capillary sprouts. These sprouts had hyperchromic endothelial and perithelial nuclei and hypertrophic cytoplasmic bodies. The new capillaries centered or surrounded granulomata. Some granulomata showed early intratubercular or peritubercular fibrosis (Fig. 6). Beneath was a dense histiocytic as well as fibroblastic granulation tissue, which housed swollen and blurred collagenous bundles. It harbored a rich network of reactive, newly laid capillaries and also granulomata.

Granulomata were more numerous and larger in surrounding areas, outside, but near the wound, under the intact skin. Here evolutive and involutive changes were intermingled. In these granulomata the histiocytes might show fibroblastic metamorphosis or congregate into syncytia or giant cells, or be interspersed with plasmacytes. Encapsulation of solitary granulomata and conglomeration of granulomata, absent in the areas uncovered by epithelium, were well represented in the para-focal areas of the granulation tissue, which were protected by the skin.

B. Controls. In the animals given nonspecific antigen there was no evidence of granulomata, of focal accumulations of histiocytes or related cells, or of endothelial or perithelial cell reactivity. The network of capillary sprouts was radially patterned. The granulation tissue appeared somewhat looser and contained fewer histiocytes than that of the experimental animals. Connective tissue fibers showed no alterative changes.

Group III. Shock Antigen in Varying Dilutions

Granulomata were found in all of the experimental animals.

There seemed to be no necessary relationship between the strength of the shock antigen, on the one hand, and the number and size of the granulomata or of their evolutive and involutive changes and their tendency to conglomerate, on the other. Early granulomata might be found in the guinea-pigs which had received undiluted shock antigen, whereas intratubercular fibrosis with or without degeneration might be seen in the guinea-pigs which had received the more diluted doses. In some fields, the histologic pictures were similar, regardless of the dilution used.

This group of experiments was designed to show graded stages of development of the granuloma. From a histogenetic approach, how-

ever, the experiment is not more adequate than the above experiments or those still to be described.

Group IV

(*A*) *Specific Antigen, Repeatedly Administered, at Time Intervals of Latency Period Length, Induces Granuloma Formation.* Granulomata, either solitary or conglomerate, were found in varying stages of involution. In some granulomata, intertubercular or intratubercular connective tissue fibrils as well as mucoid degeneration of some of the connective tissue fibers might be seen. Many granulomata displayed giant cells. Lymphocytes and plasma cells were demonstrable, especially at the junction of two or more granulomata, when the latter were incompletely coalesced. There was also plasma cell and monocytic cuffing of capillaries and precapillary arterioles, as well as perineural monocytosis. A recently superimposed mild acute inflammation with the usual marked edema and eosinophilia completed the histologic picture.

(*B*) *Nonspecific Antigen Repeatedly Administered, at Time Intervals of Latency Period Length, Re-sensitizes the Animal, Becomes Specific, and Induces Granuloma Formation.* Conglomerate granulomata were present, mostly in the stage of mucoid degeneration. There were also perivascular and perineural monocytosis and histiocytosis, without definite clear-cut granuloma-like margins. The remainder of the changes were consistent with a residual inflammation with slight, recently superimposed, acute inflammation and marked eosinophilia. Thus, the changes were identical with those seen in animals A.

Animals A received four subcutaneous injections with specific antigen, counting the original sensitizing dose of egg white. Animals B received only three injections with horse serum. The first injection of horse serum, given 10 days after the initial dose of egg albumin, became a sensitizing injection, while the doses of horse serum given on the 20th and 29th days became shock antigen. Animals B had therefore only two doses of shock antigen (whereas animals A had three) and the action of this antigen upon the focal area was 10 days shorter; yet the changes were similar in both groups.

The experiment proves that "re-sensitization" to a new specific antigen is manifested in its focal response by granulomata, and that while it is possible to annihilate a first sensitization, it still influences the histologic picture by an "additive factor," as shown by almost identical findings in both experiments.

(*C*) *Various Nonspecific Antigens, Administered at Time Intervals of Latency Period Length, but Never Repeated, Do Not Induce Granu-*

loma Formation. There was no evidence of granulomata, of focal accumulations of histiocytes and monocytes, or of endothelial or perithelial reactivity. Residual inflammation with superimposition of slight and recent acute inflammatory stages was demonstrable. There were occasional perivascular eosinophilic infiltrates.

(*D*) *Nonspecific Antigens Intercalated between Sensitizing and Shock Antigens Do Not Prevent Granuloma Formation.* There were granulomata of varying size. In most of them the histiocytes were transforming into fibroblasts. Alterative changes were conspicuous and exhibited diverse modalities ranging from hyalinization to mucoid and myxomatoid degeneration. The granulation tissue, either in the areas where it harbored granulomata, or in the areas where granulomata were absent, showed fair numbers of monocytes and histiocytes. The microscopic picture appeared "too advanced" or "too speeded-up" for an interval of time of only 2 days between the injection of a single dose of shock antigen and the killing of the animals, as well as for animals which had received in all only one shock injection. This may be attributable to a sort of "additive effect" of the previously intercalated nonspecific antigens.

III. The Anaphylactic Granuloma: Morphology, Histogenesis, Evolution, and Involution

The qualitative changes in a focal anaphylactic inflammatory response manifest themselves in two forms:

1. Histiocytic and monocytic accumulations, which may be irregularly shaped, ill-delimited, or streamer-like condensations in the granulation tissue. They are unrelated to vessels, nerves, or fat tissue. Such cell aggregates are rather rare and usually found outside the area housing the granulomata. (Diffuse histiocytosis.)

2. Round or ovoid cell accumulations, which vary in size and age, and may be solitary, or fused, encased into similar formations, or conglomerated. They are often well-delimited, and rarely faintly contoured. They may be encapsulated or not. They are related to nerves, adipose lobules, and mainly to capillaries as well as, in rarer instances, to precapillary arterioles. (Granulomata.)

(a) The perineural granulomata (Fig. 3) are generally nonencapsulated and have peripherally fading histiocytes and monocytic streamers. In cross section they appear rounded. When the nerve is cut obliquely or lengthwise, their shape is irregular, vaguely triangular, with slightly curved, internally concave margins and smoothed angles. In contradistinction to the perivascular granulomata, they are more

readily evanescent and their histiocytes are more prone to undergo fibroblastic metamorphosis. Their histiocytes and monocytes usually do not pierce the perineurium, whereas the polymorphonuclear and, especially, eosinophilic leukocytes almost always invade the nerve.

(b) Granulomata developing in and around adipose lobules (Fig. 2) constitute a variant of the perivascular granulomata, structurally modified by environmental factors. They originate at the expense of the intralobular capillaries, by multiplication and unleashing of the prospective potencies of their endothelial and perithelial cells. The newly engendered cells impinge upon the neighboring fat cells and stem, by diffuse streamers of monocytes and related cells, into the argentaffine networks which house the adipose cells. Later the reactive cells penetrate into the fat-emptied adipose cells, and the lobule is ultimately entirely converted into a granuloma. Some of the peripheral cells may spread outside the lobule into the surrounding granulation tissue.

(c) The perivascular granulomata (Figs. 1 and 4) are by far the most numerous and the most conspicuous reactive cell aggregates. They may occasionally surround a precapillary arteriole (Fig. 2). As a rule, however, the perivascular granulomata are centered, at one or another of their evolutive stages, by a functional or vestigial capillary. This capillary may be stromal, but in most instances is a newly formed capillary of the granulation tissue. In some instances, a nerve and its flanking vessels may be embedded in a mass of granulomatous cells.

Shape. A pericapillary granuloma, when solitary, is more or less regularly circular or ovoid in its outline. Reactive cells from the surrounding granulation tissue may congregate towards a nonencapsulated or already encapsulating granuloma. If they surround concentrically the former layers moulded upon the central capillary, the granuloma will be spheroid. If the reactive cells become apposed to one or both of the spheroid's poles, the shape will assume that of a spheroid surmounted by two cones and later on that of an ovoid. When cells from the granulation tissue are apposed to an encapsulating granuloma, the inner rounded nodule is incarcerated within a larger one; such encased granulomata are not uncommon. Two or more granulomata may come into contact (Fig. 6), or they may fuse together without total loss of their individuality (Figs. 7, 8, and 9). They also may form a larger or coalesced unit, the polycyclic contours of which indicate their multicentric origin. Usually granulomata show a central vestigial capillary, except when the cut surface is a secant to the spheroid granuloma, or when the central capillary has undergone complete necrosis.

Size. Most of the solitary spheroid granulomata measure about

200 μ in diameter. Ovoid single granulomata measure up to about 450 by 250 μ. The largest single granuloma found so far measured 1.5 by 0.5 mm.

Cell Constituents. Histiocytes and Derivatives. Most of the cells are histiocytes, both in actual appearance and in origin. They may show slight variations in type. Some of the cells may lack one or several of the characteristics of histiocytes or monocytes, but they come nearest to such forms. Primitive mesenchymal cells may also be found, scattered among the histiocytes of the nodule. Entirely or predominantly monocytic granulomata are frequent. In occasional granulomata, reticular cells are demonstrable. They rest upon argentaffine fibrils and harbor in their meshes monocytes, giant cells, or syncytia. The enmeshed monocytes may congregate in sheet-like fused elements.

Syncytia, Epithelioid and Giant Cells. Reactive cells, regardless of type, may form small syncytia (Fig. 8) with crowded or piled up nuclei, or larger syncytia when the nuclei are less crowded or separated. The cells may also gather into an epithelioid pattern (Fig. 8) or form giant cells (Fig. 5). Giant cells are usually found at the periphery of the granulomata.

Alterative Changes; Metamorphoses. The cell types may change with the evolutive and involutive phases of the granuloma. It is not uncommon to see them in rows in which the histiocytes which undergo alterative changes are swollen by vacuoles of varying size which give them a foamy character. Before the granulomata undergo fibrosis or degeneration, some of the histiocytes and related cells show fibroblastic metamorphosis. The peripheral histiocytes are transformed into fibroblasts earlier than the central or midzonal elements. Sometimes the marginal fibroblasts, which either delimit or start to encapsulate a granuloma, are extratubercular in origin and stem from the interstitial granulation tissue.

Lymphocytes, Plasma Cells, Eosinophils. In some instances the histiocytes of a granuloma are intermingled with other cells, such as lymphocytes, and, more often, plasma cells. The latter are peripherally located, appear in groups, parallel rows, or, more frequently, in the granulation tissue which separates solitary granulomata (Fig. 13) or incompletely fused granulomata. Lymphocytes and plasma cells usually undergo degenerative changes earlier than the histiocytes. In other instances, when several coalescing granulomata show an almost total mucoid or myxomatous degeneration, the only viable or recognizable cells present are the peripheral or intertubercular plasmacytes. In very rare instances, small granulomata may be entirely plasmacytic.

Eosinophils wander among the granuloma cells. Granulomata with no admixture of eosinophils may also be found.

Vascularization. As a rule granulomata are nonvascular, except for the central capillary, the circulatory function of which is lost very early during the histogenesis of the granuloma. In some instances a newly formed capillary courses along an arc of the tubercular periphery and indicates the limit of the granuloma. In other cases a new capillary will branch into intratubercular sprouts which never fan out beyond the outer third of the tubercular area. Penetration of newly formed capillaries into a granuloma usually ushers in fibrosis, although sclerosis may occur also in absence of an intruding vessel, by direct fibroblastic metamorphosis of the histiocytes.

Histogenesis. The initial changes are either predominantly endothelial (Fig. 11) or more markedly (Fig. 10) or even exclusively perithelial (Fig. 1). Simultaneous endothelial and perithelial responses (Fig. 12) are not uncommon and proceed with more or less identical pace in both cell types.

The endothelial cells are swollen and increase up to three to four times their usual size (Fig. 10, and more characteristic in Fig. 12). The nucleus becomes more vesicular, with peripheral, dark, rod-like chromatin strands and a rather clear center; the cytoplasm is slightly darker and in cases basophilic; the cell outlines are precise. Soon the flattened endothelial shape is lost; the cell becomes vaguely fusiform, then more rounded (Fig. 11), and finally sessile or pedunculated. It may rest anchored to the reticular wall or become detached and fall into the lumen. Mitotic figures are frequent in the metamorphosing stage of the endothelial cells as well as in their derivatives. In certain cases most of the endothelial cells respond simultaneously, although the pace of their metamorphosis is not necessarily synchronous. In other cases only one endothelial cell will display reactivity, and the remainder will be quiescent. In a short time the lumen is crowded (Fig. 11) and, in cases, plugged with cells which resemble monocytes, although their cytoplasm may be larger than that of the typical monocyte and the nucleus less indented. Some of the monocytoid forms have cytoplasmic processes. The derivatives of the endothelial cells may be flanked by intraluminal eosinophils. They may phagocytize erythrocytic débris, although this is only rarely seen. Red cell or hemoglobin engulfment, when it occurs, is imperfect or clumsy. No fibrinous thrombi are seen at this or any other stage in capillaries, but may be seen in postcapillary venules. The clump of intraluminal cells does not elicit at any stage a parietal fibroblastic reaction, nor does it

undergo any abortive, involutive, or degenerative process prior to granuloma formation.

The perithelial cells likewise show mitotic figures (Fig. 10) and more frequently than the endothelial cells. Clumps of cells appear at points where usually only one pericyte is expected. The newly formed cells are piled up, sometimes in an epithelial-sheet fashion. The forms are not clear-cut at the beginning; and their evolution, step by step, is more difficult to follow than in the case of the endothelial derivatives. Although they may not be typical histiocytes, they resemble most closely this type, as they display round to flattened cytoplasmic bodies with vaguely ragged outlines and bean-shaped nuclei with inconspicuous nucleoli. Likewise some cell derivatives come nearest to monocytic or original mesenchymal forms, but cannot in all instances be indisputably termed as such. Histiocytic and monocytic patterns predominate, however.

Concomitantly with the endothelial and perithelial metamorphoses, the capillary wall undergoes changes of its own. The various stages range from irregular, blurred staining, fragmented straight or wavy fibril-like structures to a dot-like disintegration. In some cases, when the capillary walls have disappeared, the cells of endothelial ancestry can still be differentiated from those of perithelial origin, the former, mostly monocytic in type, being encased by the latter, which are mainly histiocytic. In subsequent stages, in most instances, this differentiation is no longer possible.

When cell aggregates surround fibrillar remnants of a capillary wall the impression may be gained that the anaphylactic granuloma may have an origin similar to that of an Aschoff body. This impression is false, however. Indeed, (1) all stages ranging from centering capillaries that are intact to capillary wall débris are demonstrable; (2) the dot-like disintegrated walls of the central capillary are reticular and not collagenous; (3) these remnants do not swell, but disintegrate; (4) they do not undergo a fibrinoid degeneration; (5) the reactive cells of a granuloma, even at their earliest evolutive stages, do not show bar-like central nucleoli, conspicuous nuclear vesiculation, or markedly ragged cell outlines; (6) the granuloma at any stage shows more compactness of texture and cell richness than an Aschoff body; (7) the intracapillary and pericapillary origin of a granuloma is in most cases clearly evident in younger granulomata, and traceable in retrospect in older ones.

At this stage of its formation the granuloma is made up of crowded cells, arranged either concentrically or in no definite patterns. Although no marginal fibroblasts are as yet present, the granuloma has a

clear-cut outline, except when it sends out cell streamers. But even in such cases, the bulk of the cell aggregate constitutes an anatomic unit.

At various stages of its development, the granuloma acts as a center of attraction for neighboring cells when these are endowed with identical morphologic and functional characters. Histiocytes and monocytes from the surrounding granulation tissue appear to have pivoted and turned one of their poles towards the periphery of the nodule, to have come nearer to it, and finally to have reinforced the cells of the granuloma.

Encapsulation, Fibrosis, Involution. Involutive changes may be seen at any time, even when the granuloma shows signs of growth. One or several histiocytes, whether peripheral or midzonal, may undergo a fibroblastic metamorphosis. This is heralded by a change in their axis of orientation, which from radial becomes tangential, or by their coursing in a curvilinear direction, with their concavity facing the center of the granuloma. They may lie in single files or in concentric layers, be localized on only one of the sectors of the circumference, or be scattered all around the periphery of the granuloma (Fig. 6). They may or they may not be accompanied by connective tissue fibrils. Encapsulation may therefore be only polar or on an arc of the circumference before becoming circular. In many cases, in which the wrapping collagenous fibers are missing, the encapsulation is only apparent and rather in the nature of a fibroblastic delimitation. Fibroblastic metamorphosis of midzonal histiocytes brings about a delineation of a small circular arc which finally becomes a spheroid encased into the granuloma. Sometimes encapsulation is begun, but new histiocytes which come from the granulation tissue are apposed to the fibroblastic barrier. In the course of time, the granuloma will be either partially or entirely fibrosed.

Fibrosis may or may not be followed by degeneration. This degeneration may be mucoid (Fig. 8) or myxomatoid (Fig. 9). In the mucoid type the fibrils, and later on the cell processes and the fibroblastic bodies as well, take the basic components of the Mallory stain and assume afterwards a greenish hue (pale orange with phosphotungstic acid hematoxylin). Subsequently parts of the granuloma are replaced by greenish patches where vestigial cells are seen, either shriveled or swollen. In the myxomatoid type the cells become separated but are still in contact with their polar or stellate processes; the intervening matrix is ridged, or coarsely granular, or homogeneously swollen and of the same greenish hue (when stained with Mallory's phloxine and methylene blue). The picture suggests Wharton's jelly.

Degenerating granulomata may show a diversified structure. In the

mottled greenish mass of mucoid matrix, the cells may or may not be identifiable. In other cases the degeneration is preceded by the appearance of giant cells or syncytia. Fibrosed or degenerated, the granulomata are always recognizable by their shape, by the concentric or vaguely whorled arrangement of their cell remnants, and by the richness of the circumscribing granulation tissue in histiocytes and plasma cells.

No experiments have been performed to ascertain the length of time necessary for a granuloma to disappear.

Summary

Subcutaneous injections of shock antigen into specifically sensitized guinea-pigs induce a local inflammatory reaction which is at variance with nonanaphylactic ("normergic") inflammation, both quantitatively and qualitatively.

1. The quantitative changes are known from the work of Arthus and Breton, Rössle, Gerlach, and others, and consist of a stormy and intense unfolding of the process with initial ischemia, overwhelming edema, marked polynucleosis, eosinophilia, and early fibrosis as main characteristics.

2. The qualitative changes are characterized by:

A. Complete local resorption of the specific antigen. Whether this resorption is in the nature of a local fixation by either edema or reactive histiocytes is, at this stage of the investigation, only conjectural.

B. A histiocytosis, which may manifest itself:

I. Rarely in diffuse aggregates of histiocytes and related cells.

II. Most frequently by granulomata, which are:

(a) Perineural, the least reactive cell aggregate;

(b) Perivascular, and especially pericapillary, the best represented and the most reactive cell aggregate;

(c) Intra-adipose and peri-adipose, an environmentally conditioned variant of the pericapillary granuloma.

3. The morphology, cell constituents, histogenesis, evolution, and involution of the anaphylactic granuloma have been described.

4. The origin of the granulomata is twofold:

A. Endothelial and/or perithelial, through prospective potencies of these cells.

B. In a minor degree, through apposition of histiocytes from the surrounding reactive granulation tissue.

5. The qualitative reaction occurs constantly. Dilution of the shock antigen is immaterial. There are, however, variations with regard to

the intensity of the reaction (number, size of granulomata, their tendency to conglomerate) and to its modalities (presence or absence of plasma cells; epithelioid patterns; giant cells). This variability in intensity and modality of the focal anaphylactic response is at present not attributable to any factor. Such variations may be seen from experiment to experiment, or in cases, from animal to animal within the same experimental group, and even from tissue block to block.

6. The part played by intermingled cells (lymphocytes, plasma cells) is not clear. However, plasma cells are more frequently seen: (a) at the periphery of the granulomata, especially when the nodules fuse or congregate; (b) at the periphery of fibrosing and, especially, degenerating granulomata; (c) in granulomata induced by shock antigen repeated several times at long intervals; (d) in pericapillary mantlings; (e) occasionally as small plasmacytic granulomata.

7. Local injections of nonspecific antigen do not induce granulomata, except when the nonspecific antigen, by repeated injections, at time intervals of latency period length, re-sensitizes the animals and becomes specific (or shock) antigen.

8. Nonspecific antigens, when preceding specific antigens, or when intercalated between the sensitizing and shock antigens, may speed up the histogenesis, evolution, and involution of granulomata, and act as "additive factors" which elicit "additive effects."

9. The anaphylactic granuloma is not patterned histogenetically and histologically after the Aschoff body of rheumatic fever.

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DESCRIPTION OF PLATES

PLATE 140

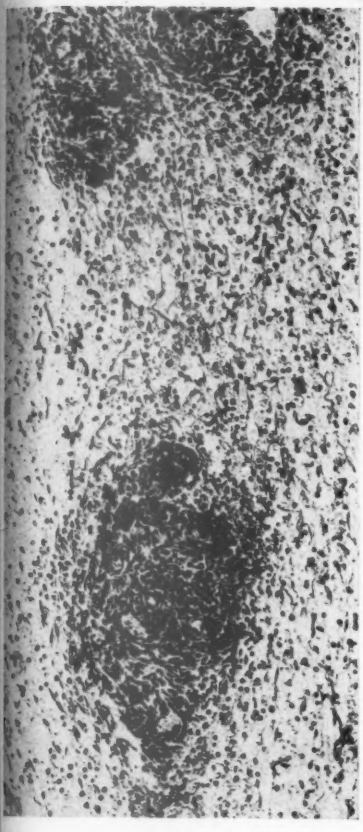
FIG. 1. Group I, A. Three granulomata, with intervening area of edema with monocytic infiltration. The upper left granuloma is centered about an arteriole. The lower, although appearing as an anatomic unit, is multicentric. Of note are the widely dilated capillaries and the absence of endothelial reactivities; this is an example of a granuloma of exclusively perithelial origin. Phloxine and methylene blue stain. × 140.

FIG. 2. Group I, A. Peri-adipose and intra-adipose granuloma, early. The lobule is not yet entirely converted into a granuloma. Next to it (separated by lymphatics) two coalescing peri-arteriolar granulomata can be seen. Phloxine and methylene blue stain. × 140.

FIG. 3. Group I, A. Granuloma embedding a nerve and its vascular satellites. Of note is the appositional growth. Phloxine and methylene blue stain. × 140.

FIG. 4. Group I, A. Well outlined, but nonencapsulated granuloma. Phloxine and methylene blue stain. × 140.

FIG. 5. Group IV, A. Small granulomata converted into groups of giant cells. Phosphotungstic acid hematoxylin stain. × 460.



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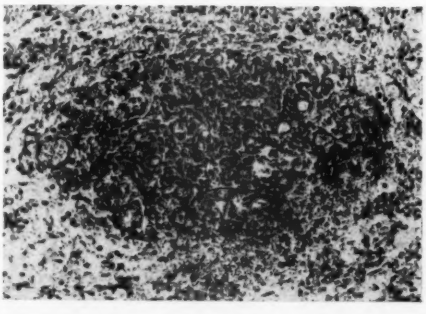

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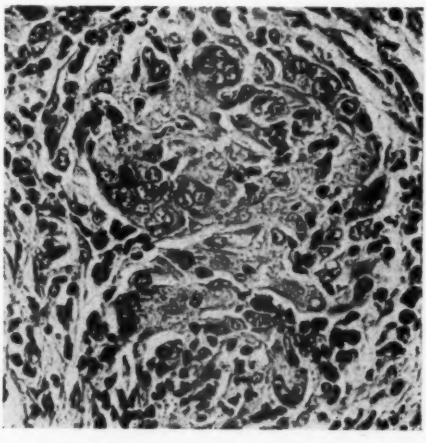

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Granuloma in Focal Anaphylactic Inflammation

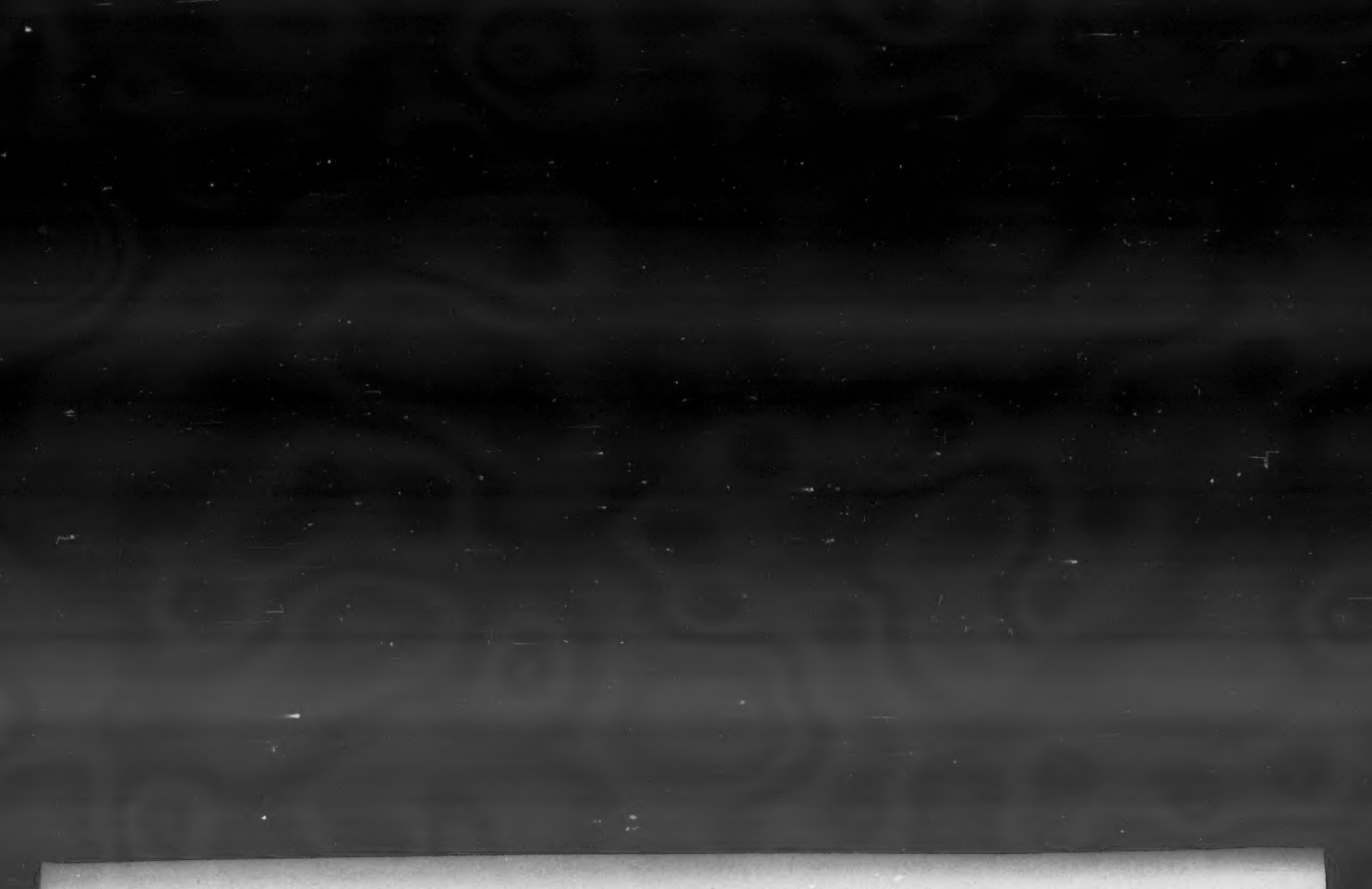
Plate 141

Fig. 6. Group II, A. Group of granulomata with fibrosing internodular granulation tissue. The upper (and leftwards directed) granuloma shows the vestigial capillary with obliterated lumen (group of whorling cells). In all granulomata there is evidence of actual or impending fibroblastic metamorphosis. Masson's trichrome stain. × 555.

Fig. 7. Group IV, A. Histiocytic granulomata, with compact texture. Phosphotungstic acid hematoxylin stain. × 595.

Fig. 8. Group IV, A. Granulomata and internodular granulation tissue. Upper granuloma made up mostly of histiocytes (fibrillar structure of cytoplasm brought out by Mallory's phosphotungstic acid hematoxylin stain). Vestigial capillary is slightly eccentric. In lower granuloma, where an early, central mucoid degeneration is demonstrable, the marginal histiocytes show an epithelioid pattern and formation of syncytia. Of note is the perinodular fibrosis. Phosphotungstic acid hematoxylin stain. × 595.

Fig. 9. Group II, A. Group of degenerating granulomata. Myxomatoid degeneration (best seen in the granuloma on the left). Phloxine and methylene blue stain. × 280.



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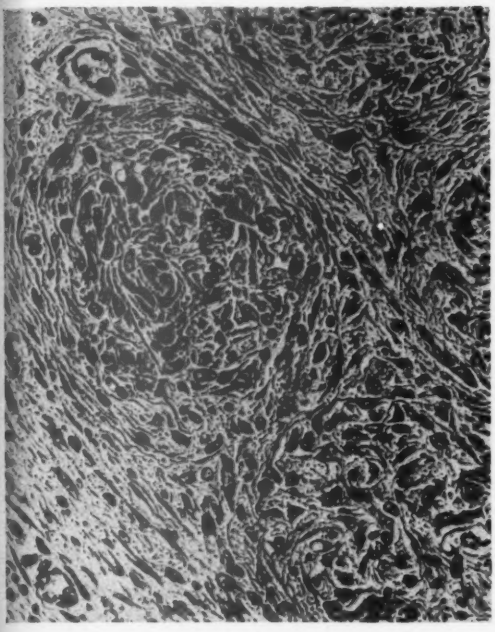

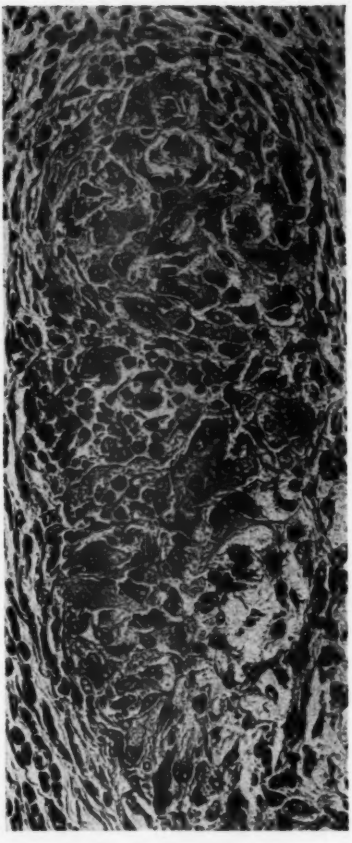

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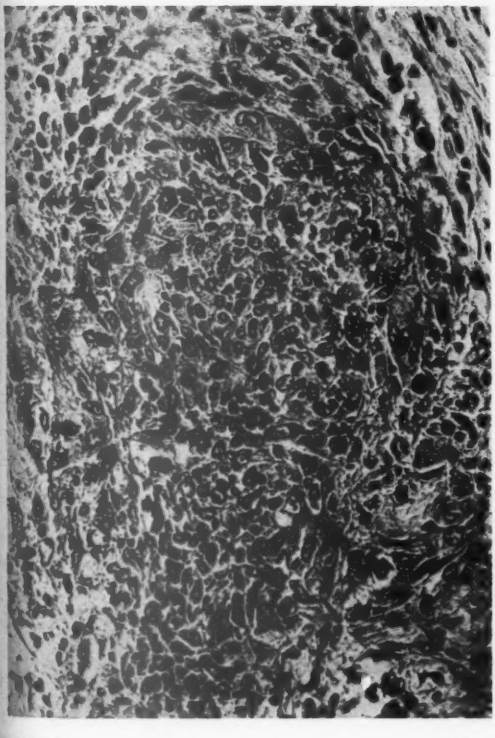
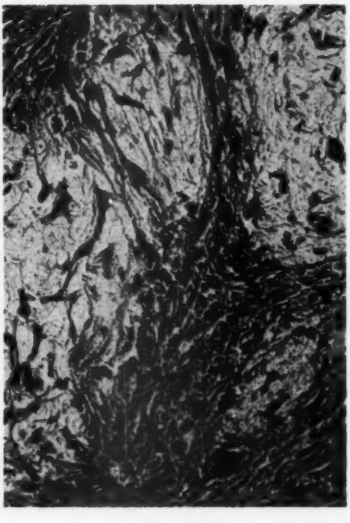




9

Goddard Granuloma in Focal Anaphylactic Inflammation

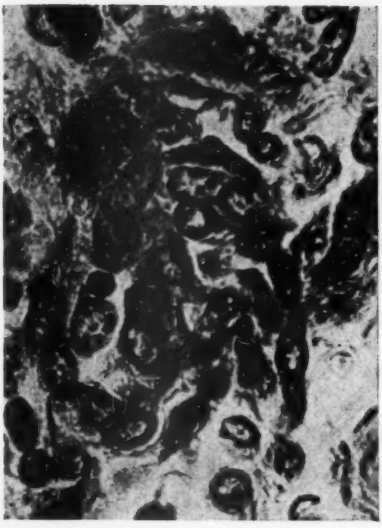
PLATE 142

FIG. 10. Group I, A. Histogenesis of a pericapillary granuloma. Marked perithelial proliferation (several mitotic figures) and metamorphosis (histiocytes, monocytes). Earliest endothelial hyperplasia. Phloxine and methylene blue stain. × 1110.

FIG. 11. Group I, A. Histogenesis. Section through an S-shaped capillary, showing narrowing of the lumen and endothelial hyperplasia, hypertrophy, and metamorphosis. There is also slight perithelial reactivity. Phloxine and methylene blue stain. × 1110.

FIG. 12. Group I, A. Two neighboring capillaries. The capillary towards the left of the figure shows slight to moderate endothelial and perithelial reactivity, but no luminal blockage. The perithelial cells (left margin) are still metamorphosing. The capillary on the right no longer shows its reticulum wall; only a few endothelial cells can be identified; the lumen is crowded with metamorphosing cells. In the lower sector of the figure a vaguely triangular to trapezoidal patch shows the derived cells at almost the end-stage of their metamorphosis. Phloxine and methylene blue stain. × 1110.

FIG. 13. Group IV, A. Plasma cell infiltrate in intertubercular granulation tissue. In places the circular arrangement may give the false impression of plasmacytic granulomata. Phloxine and methylene blue stain. × 742.



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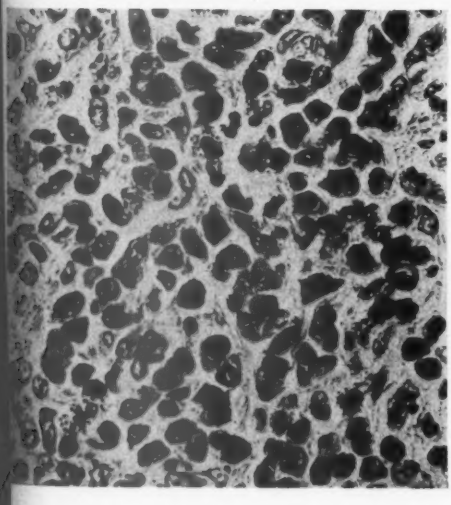

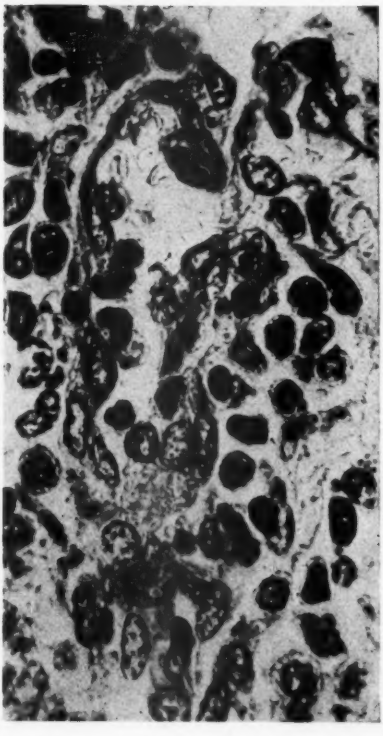

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Granuloma in Focal Anaphylactic Inflammation

HEMOGLOBIN CRYSTALS, CASTS, AND GLOBULES IN THE RENAL TUBULES OF GUINEA-PIGS FOLLOWING CHEMICAL HEMOLYSIS*

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In 1840 Hünefeld [1] observed that bright red plate-like crystals with sharp borders were deposited when the blood of an earthworm was exposed between glass plates. In addition, he referred to crystals from the blood of man and of the pig. Later, in 1849, Reichert [2] reported finding tetrahedral crystals in the fetal membranes and in the mucous membrane of the uterus of a guinea-pig which had died suddenly, and which was examined 6 hours after death. There were four fetuses in the uterus, and in all four placentas the crystals were found. Interest was aroused and reports of other work followed. One of the greatest advances was made by Funke,[3] who in 1851 devised methods of preparing crystals and suggested that the crystals of hemoglobin showed certain physical characteristics which seemed to be related to species. In the next 50 years there were numerous contributions.

In 1911 Miller [4] described crystalline forms of hemoglobin in the capsular space of the glomeruli in a man dying of arsine poisoning. In the same kidney somewhat more delicate crystals were found "in the vicinity of a large artery." He noted in a review of the literature that hemoglobin crystals had been described in the kidneys of dogs and rabbits following poisoning with hemolytic agents or scalding. Since the form of hemoglobin crystals seemed to be different in individual species, including man, he presumed that these differences were explained by the easier crystallization of hemoglobin in many animals, as the horse, dog, and rabbit. This was very soon after the publication of Reichert and Brown [5] in 1909, in which they demonstrated the species specificity of these hemoglobin forms.

Recently one of us (R. C. D.) [6] noted briefly the occurrence of tetrahedral crystals of hemoglobin in the renal tubules of guinea-pigs following chemical hemolysis. Since then these characteristic crystals have been found in many animals of this species, and their staining qualities have been further studied by means of several hemoglobin stains.[6-8]

We have also observed hemoglobin crystals in the renal tubules of rats following massive hemolysis and in rabbits ‡ after experimental

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† Now at U.S. Marine Hospital, Baltimore, Md.
‡ Courtesy of Dr. R. D. Lillie.

scald burns; and also in the lumen of a hemorrhagic, obstructed uterus * of a rat, and in an intrathoracic lymph node of a mouse. In these species hemoglobin crystals are identified with difficulty because of their complicated structure, but in guinea-pigs they are recognized with ease by their distinct tetrahedral form.

Studies on the mechanism of hemoglobinuria, the renal threshold for hemoglobin, and resorption of this substance by the epithelium of the convoluted tubules have been reported.[9,10] That the amount of fluid in the renal tubules may determine the deposition of hemoglobin has been discussed by Lison.[11]

Methods and Observations

In this study, 97 guinea-pigs were subjected to a *single 1-hour exposure* to stibine-air mixtures. Stibine (SbH_3) is a highly toxic gas with hemolytic properties similar to those of arsine (AsH_3).[12] The stibine was prepared by the interaction of a magnesium antimony alloy and dilute hydrochloric acid and the gas mixtures were analyzed for antimony by the rhodamine B method.[13] The guinea-pigs were exposed singly or in groups. While the concentration of stibine was held to fairly close limits during any one exposure, the concentrations employed in the series of exposures ranged from 44 to 293 parts per million (p.p.m.).

Death rarely occurred after exposure at or near the 44 p.p.m. level, but was frequent at 90 p.p.m. or greater. The survival times for animals dying from stibine intoxication varied between 45 minutes and 19 days after exposure. In order to study further the pathologic changes taking place at various time intervals, many of the exposed animals were sacrificed, thus extending the post-exposure time up to 5 weeks and, in a few cases, up to nearly 3 years.

In guinea-pigs succumbing from stibine intoxication during the period of from 2 to 6 days following exposure, the usual clinical findings before death were: dyspnea, lassitude, weakness, anorexia, often hemoglobinuria and a shock syndrome including subnormal temperature and decreased peripheral circulation, and finally coma.

In general, the higher the concentration of stibine, the sooner the beginning and the longer the duration of the hemoglobinuria after exposure. It was observed as early as 45 minutes, usually disappeared within 2 days, and was never found to persist longer than 4 days. During much of the time in which hemoglobinuria was present, hemoglobinemia also occurred, as shown by positive spectroscopic tests for

* Courtesy of Dr. A. A. Nelson, Pathologist, U. S. Food and Drug Administration.

oxyhemoglobin in the plasma. While the presence of hemoglobin in the urine was determined routinely by the benzidine test, this was often checked spectroscopically. Likewise, frequent microscopic tests showed that hemoglobinuria and not hematuria occurred in these animals.

Since hemoglobinuria usually was not present at concentrations below 66 p.p.m. of stibine, 21 guinea-pigs of the experimental animals exposed to less than this concentration are excluded from consideration at this time. Therefore, our main interest centers on the other 76 animals exposed to 66 p.p.m. and above. It should be noted, however, that hemoglobinuria can and has been produced under unusual conditions in a high proportion of guinea-pigs at concentrations less than the above value.

Some of the animals exposed to 66 p.p.m. or more were anuric; in others, experimental conditions did not always permit collection of urine before death. Of the 61 animals from which urine was tested during life, 49 (80 per cent) exhibited hemoglobinuria. On the basis of the benzidine test, the amount of hemoglobin in the urine was marked (4 plus) in about two-thirds of the animals and moderate or slight (3 plus to 1 plus) in the others.

The presence of hemoglobin in the renal tubules is manifested in one of three forms, namely, crystals, casts, or globules, the first two in the lumen and the last in the cytoplasm of the epithelial cells. These hemoglobin deposits were identified by means of their tinctorial reactions in the tissues (Figs. 2 to 6).

The form and tinctorial qualities of these crystals are similar to those of hemoglobin crystals precipitated from the blood of guinea-pigs by a slight modification of the method of Reichert and Brown.[5] Crystallized from the mother liquor by slow evaporation under a coverslip, the preparations showed tetrahedral crystals, together with truncated and twinned varieties * (Fig. 1).

Hemoglobin crystals were found in the renal tubules of only 23 of 40 guinea-pigs dying or killed during the second to sixth days inclusive, following exposure to 66 p.p.m. or more of stibine (Table I). Of these 40, 23 had both hemoglobin crystals and hemoglobin casts, 3 had casts without crystals, and 14 had neither casts nor crystals, although 2 showed hemoglobin globules. One of the 14 animals, showing only 2 or 3 poorly formed hemoglobin casts, was considered as having none.

Detailed description of the histopathologic findings in the kidneys will be limited to this group of 40 animals, since in the remaining 57

* According to Reichert and Brown, crystals of guinea-pig hemoglobin, although appearing to be tetrahedral, have been shown to be orthorhombic sphenoidal in form.

guinea-pigs in this study hemoglobin deposits were seldom found, the incidence for crystals, casts, and globules being 0, 6, and 6 animals, respectively.

Pathologic Findings in the Group of 40 Animals

Upon gross examination the kidneys had the usual appearance associated with intravascular hemolysis. The external as well as the cut surface was dark red, and fine to coarse blackish red striations were often seen in the lighter, outer medullary zone. Occasionally, punctate

Table I

Association of Hemoglobin Crystals and Hemoglobin Casts with Hemoglobinuria in 40 Guinea-Pigs Dying or Killed during the 2 to 6 Day Period Following Exposure to 66 Parts per Million or More of Stibine

				Hemoglobin crystals in the renal tubules					Hemoglobin casts in the renal tubules				
	Animals in each group			Number of males		Number of females			Number of males		Number of females		
Hemoglobinuria	Male	Female	Total	Present	Absent	Present	Absent	Total	Present	Absent	Present	Absent	Total
Absent	0	3	3	0	0	0	3	3	0	0	0	3	3
Present	19	8	27	12	7	5	3	27	13	6	6	2	27
Not determined*	9	1	10	6	3	0	1	10	7	2	0	1	10
	28	12	40	18	10	5	7	40	20	8	6	6	40

* Some animals in this classification were anuric; in others, experimental conditions did not permit testing of urine.

blackish red areas were found on the surface under the stripped capsule.

Microscopically, crystals were found in 23 of the 26 guinea-pigs with casts. On the basis of a single 5 μ routine section of tissue fixed in phosphate-buffered neutral formalin and stained by the hematoxylin-picric acid method,[6] each of the 23 animals showed single or grouped crystals in the lumina of 2 to 36 tubules. Using this standardized method, the average number of crystal-containing tubules per animal, arranged according to days (from the 2nd through the 6th), was as follows: 12, 16, 7, 8, and 9.

These crystals had the same tinctorial reactions as the erythrocytes in nearby blood vessels and averaged in over-all length about 20 μ, with extremes of 5 to 54 μ. In formalin-fixed tissue they characteristically had triangular configurations and showed perceptible depth and sharp straight edges (Figs. 2 to 5). However, occasionally it was possible to demonstrate their tetrahedral form. Frequently the edges

showed grooves or lacunae containing a cell or two of mononuclear or polymorphonuclear neutrophilic type (Fig. 6). These cells often formed a part of a conglomerate mass of similar cells that partially or completely enveloped a crystal.

The crystals appeared to be mainly in the convoluted tubules and the loops of Henle in the outer medullary zone. In 2 animals they were noted also in two or three large collecting tubules in the papilla. None was found in the identifiable straight collecting tubules of the medullary rays, and none was found in glomerular spaces.

There was some variation in the number and location of the tubules containing crystals, in relation to the time of death. Arranged in order of successive days following exposure, the ratios of crystal-containing tubules in the cortex (convoluted tubules) to those in the outer medulla (Henle's loop) were as follows: 6:1, 4:1, 4:3, 1:1, and in the single animal dying on the 6th day the medulla was not well seen. The greatest concentration of crystals occurred in the 3rd day group.

In addition to the crystals, hemoglobin casts (Figs. 3 to 6) were found in 26 of the 40 animals in the 2 to 6 day group. The average number was greatest in the 3rd day group, and they were located chiefly in the convoluted and loop tubules and, rarely, in the large collecting tubules. There was no consistent chronologic relationship between the number of hemoglobin casts found in the tubules of the cortex and the number in the tubules of the medulla.

In the 2nd and 3rd day group of guinea-pigs there were small to large numbers of cytoplasmic hemoglobin globules (Fig. 5) in the convoluted tubular epithelium of 12 of the 18 animals; in the other 22 animals, dead on the 4th to 6th days, only 2 had globules. Small to large amounts of hemosiderin were noted in the cytoplasm of the epithelium lining the convoluted tubules of 37 of the 40 animals, the numbers and the sizes of the granules being slightly greater in the last 3 days.

Dilatation of the tubules was noted in 33 of the 40 animals. In 14, only the tubules of the medullary rays were dilated; in 19, tubules in both cortex and medullary rays were involved. Cytoplasmic basophilia was noted in many of the tubules showing considerable dilatation.

Coagulation necrosis of the epithelium lining convoluted tubules was noted in about half of the 26 animals, the number of affected tubules ranging from about 1 to 5 per section in scattered animals among the series, to a maximum of 20 to 30 in 4 animals in the 3rd and 4th day groups. This necrosis was frequently seen in epithelial cells packed with hemoglobin globules, though not as an invariable accompaniment, and was often found in tubules containing hemoglobin casts or cellular

casts. Occasionally the necrotic cells, along with granular and fibrillar débris, were free in the lumen.

In 23 of 40 animals cellular casts were noted in a few convoluted tubules. In the midst of closely packed mononuclear cells and occasional polymorphonuclear neutrophils, a hemoglobin cast or crystal was often embedded. These cells were often found lying in marginal grooves and, rarely, appeared to be within the substance of the casts or crystals.

In the 26 animals with hemoglobin casts, finely to coarsely globular intracellular fat was noted in large amounts on the 5th and 6th days, but on the 2nd to 4th days there were only traces or small amounts. In the 14 showing no hemoglobin casts there were usually moderate to large amounts of fat on the 3rd through the 6th days, and only traces on the 2nd day. The fat was found mainly in the epithelium of the convoluted tubules and Henle's loops.

Positive evidence of glomerular change was found in but 5 animals. In 2, many of the glomerular tufts were shrunken and bloodless; in 2 others, 6 or 8 glomeruli revealed several capillaries dilated and plugged by dense eosinophilic masses resembling compressed erythrocytes and granular hemoglobin. In the fifth, the glomerular spaces of at least four-fifths of the renal corpuscles were markedly dilated, and in a few instances the first portion of the proximal convoluted tubule was also greatly expanded. It was in this animal that the greatest degree of tubular dilatation was noted.

Pathologic Findings in the Kidneys of the Remaining 57 Animals

Examination of the other 57 animals in this study was carried out in somewhat less detail since hemoglobin deposits were so seldom found. Of this number, hemoglobin globules were found in only 2 of the 21 guinea-pigs exposed to concentrations of stibine below 66 p.p.m.; this occurred in those killed on the first day. Hemoglobinuria and casts and crystals were absent in all 21, but hemosiderin was noted in 5. Traces to small amounts of fat were found in 4 of the 10 animals examined.

The remaining 36 animals, not previously discussed, consisted of those exposed to more than 66 p.p.m. of stibine and killed or dying outside the 2 to 6 day period. Of this group, 11 were dead on the first day. Hemoglobinuria was present in 7 of the 8 animals from which urine was obtained. The incidence of hemoglobin crystals, casts, and globules was 0, 3, and 4, respectively. In addition, hemosiderin was found in traces to small amounts in 4 animals, and in moderate amount

in one more. Traces to small amounts of fat occurred in 6 of the 8 animals studied.

The residual 25 guinea-pigs, having survival times of from 7 days to nearly 3 years, showed neither hemoglobin crystals nor globules and only 3 had hemoglobin casts. Fourteen of the 20 animals tested had had hemoglobinuria. Among this group of 25, hemosiderin was found in small amounts in 20, and in moderate amounts in 2 animals. Fat was found in traces to large amounts in 11 of the 17 guinea-pigs examined.

Discussion

Considering the whole group of 97 animals in the study, only after exposure to high concentrations (92 p.p.m. or greater) of stibine was hemosiderin found in moderate amounts in the kidneys of guinea-pigs having survival times of from 3 to 5 weeks. At concentrations lower than this, the hemosiderin was either absent or present in traces.

Animals exposed to concentrations of stibine in excess of 133 p.p.m. usually survived for only a few hours. These generally had hemoglobinuria and sometimes showed hemoglobin casts and globules but no crystals were found under these conditions.

It may be assumed that when hemoglobin is present in the renal tubules, it must become concentrated by some means before crystallization can occur. It is reasonable, likewise, to assume that the structures which we recognize as casts are masses of highly concentrated hemoglobin. The physicochemical state of the hemoglobin in the casts in the 2 to 6 day group of animals evidently fulfills the conditions necessary for crystallization, for there were 23 of the 26 animals with casts (88 per cent) which had crystals.

However, hemoglobin crystals were not always found when hemoglobin casts and hemoglobinuria were present, even in the 2 to 6 day group, for 2 animals had both hemoglobin casts and clinical hemoglobinuria but showed no crystals; and in addition, one animal, in which the ante-mortem urine was not tested for hemoglobin, had casts but no crystals. These 3 animals apparently represent a minority in which the specific physicochemical conditions for crystallization were not present in the tubules. None of the other 14 of the 40 in the 2 to 6 day group had either hemoglobin casts or crystals.

Of the remaining 57 of the 97 animals in this study, 21 were exposed to concentrations of stibine less than 66 p.p.m. Among these hemoglobinuria did not occur and no hemoglobin casts were found, although two showed hemoglobin globules. The residual group of 36 guinea-pigs, dying outside the 2 to 6 day period, had exposures between 66 and 293 p.p.m. of stibine, frequently had hemoglobinuria, and occasionally

exhibited hemoglobin casts. However, in none of these 57 animals were crystals found, regardless of time of survival.

These findings suggest the possible relationships between the concentration of the inhaled hemolytic agent, the survival time, and the deposition of hemoglobin casts and crystals in the kidney. Assuming that hemoglobin crystals cannot form in the renal tubules unless hemoglobin is present in the lumen, there should be close correlation in the incidence of hemoglobinuria, hemoglobin casts, and hemoglobin crystals. These relationships may be observed in Table I. Here in the 2 to 6 day post-exposure group in which hemoglobinuria was present, a total of 27 guinea-pigs, one notes that there were hemoglobin crystals in the renal tubules of 17. In 6 other animals, in which the benzidine test was not done, there were crystals associated with casts. In 3 other guinea-pigs, in which hemoglobinuria was absent, there were neither casts nor crystals. However, one of these showed hemoglobin globules in the renal epithelium.

From a consideration of the data for the 97 animals in this study it is possible to formulate a picture of the events which occur as the animals are exposed to increasing concentrations of the hemolytic gas. The dependence both on concentration and time can be seen from Table II which summarizes the clinical and histopathologic findings with reference to hemoglobinuria and associated phenomena.

The effect of the concentration of the hemolytic gas and the duration of hemoglobinuria is dependent on so many variable factors that it is difficult to predict the amount of hemolysis that is likely to occur in any one animal, or whether hemoglobinuria will be present. There are several generally recognized contributory factors which have a bearing on the deposition of hemoglobin in the kidney during hemolysis, among which are pH of the urine, renal threshold for hemoglobin, and degree of diuresis. The formation of crystals in scattered tubules of the kidney points to a widely variable physicochemical environment in different nephrons during hemoglobinuria. Localized crystal formation in scattered areas in a wet drop, when using the microchemical triple nitrite test for lead,[14,15] presents an interesting analogy.

The relatively great solubility of crystalline guinea-pig hemoglobin may explain why these crystals were never found in the kidneys of these animals beyond the sixth post-exposure day and also why the crystals were so small and frequently appeared to have irregular edges, as if they were in the process of dissolving.

Using stibine as the hemolytic agent, attempts[16] made to demonstrate the presence of crystalline hemoglobin in the kidneys of several other species, such as rats, mice, rabbits, dogs, cats, and monkeys, have

been only occasionally successful. Hemoglobinuria was absent in the rabbit, dog, cat, and monkey; it persisted in the rat for only a few hours; and when present in mice invariably resulted in quick death of the animals. It is believed, therefore, that neither the time nor the concentration was of sufficient magnitude to insure crystallization in the kidneys of mice and rats.

TABLE II

Summary of Clinical and Histopathologic Findings with Reference to Hemoglobinuria and Associated Phenomena

Concentration of hemolytic gas (SbH_3) in parts per million	Presence of hemoglobin in urine	Presence of hemoglobin globules, casts, and crystals in kidneys
44–65	Very infrequent	Globules: infrequent Casts: absent Crystals: absent
66–91	Frequent	Globules: frequent in 1–3 day group; infrequent thereafter Casts: infrequent Crystals: absent
92–133	Almost invariable	Globules: very frequent in 2–3 day group; infrequent thereafter Casts: present in about ¾ of 0–6 day group; about ¼ of 6–9 day group; infrequent thereafter Crystals: present in about 9/10 of 2–6 day group; absent before or after that time

SUMMARY

Single one-hour exposures to stibine, a hemolytic gas similar to arsine, were administered to guinea-pigs, wide ranges of concentration being used with different groups of animals. Hemoglobinuria was observed frequently with concentrations above 65 parts per million.

Under conditions which are described, hemoglobin crystals were formed in the renal tubules of 23 of 97 guinea-pigs. The crystals were found only in animals dying or killed on the second through the sixth day following the exposure to 92 p.p.m. or higher. All of the animals showing crystals had clinical or microscopic evidence of hemolysis, or both. In guinea-pigs succumbing during the above time interval, the usual clinical picture before death was that of dyspnea, weakness, often a shock syndrome, and hemoglobinuria followed by coma.

In addition to crystals, many of the tubules showed hemoglobin casts and globules. The association of these three forms in relation to time and stibine concentration is indicated. Other pathologic conditions, such as tubular dilatation, coagulation necrosis, cellular casts, fat, and glomerular changes, were sometimes noted. Hemosiderosis in

the kidney, although of frequent occurrence, was not found in animals with 3 to 5 weeks of survival time except after exposure to high concentrations of stibine.

There are factors, other than stibine concentration, which may influence the deposition of one or more forms of hemoglobin in the tubules. The data presented are sufficient to give a picture of the chronologic development of hemoglobin globules, casts, and crystals in relation to concentration and time.

Acknowledgment of technical assistance in this work is due Mr. Ervin J. Liljegren, Mr. David J. Zimmer, and Mr. Edwin C. Thompson, all of the Laboratory of Physical Biology, National Institute of Health, Bethesda, Md.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 143

FIG. 1. Preparation of guinea-pig blood showing tetrahedral crystals together with truncated and twinned varieties. × 310.

FIG. 2. Small to medium-sized hemoglobin crystals suspended in fibrillar material in the lumen of a dilated tubule. Of note are the two small triangular shaped crystals apparently in the cytoplasm of an epithelial cell lining the tubule at one extremity of the sectioned tubule. × 850.

FIG. 3. Hemoglobin crystals in groups, twinned forms, and singly in one renal tubule. Two hemoglobin casts can be seen, one cut transversely and the other longitudinally. The latter shows thinned, irregular, ragged edges at one end due to beginning resolution. × 390.

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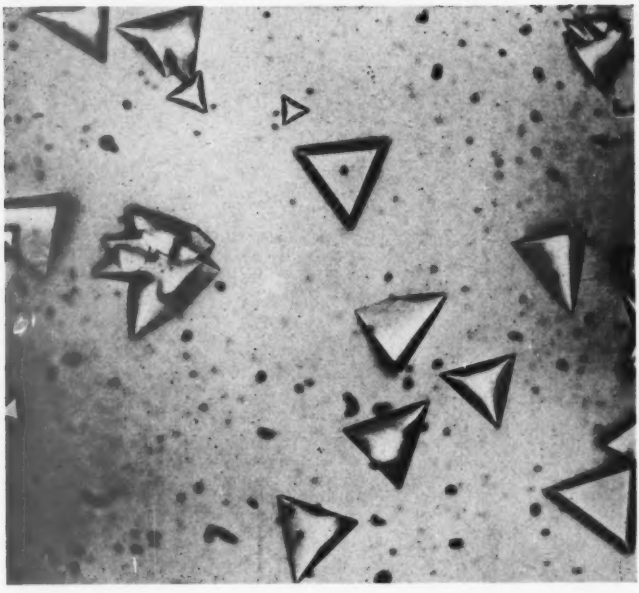

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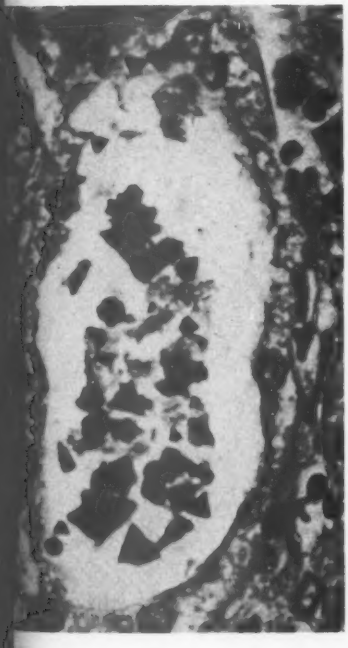

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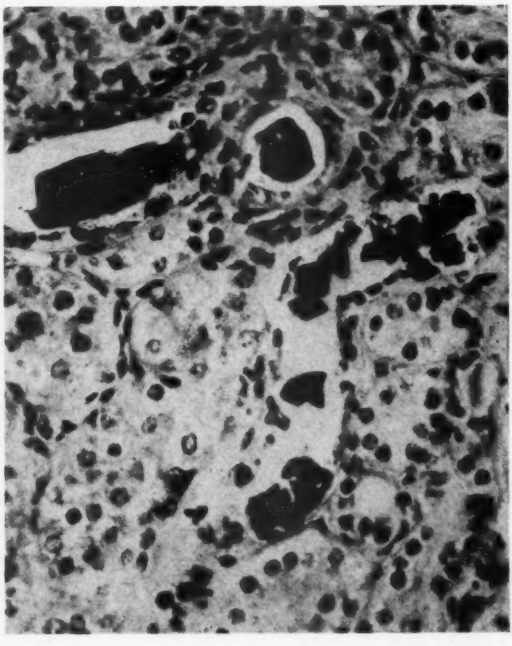

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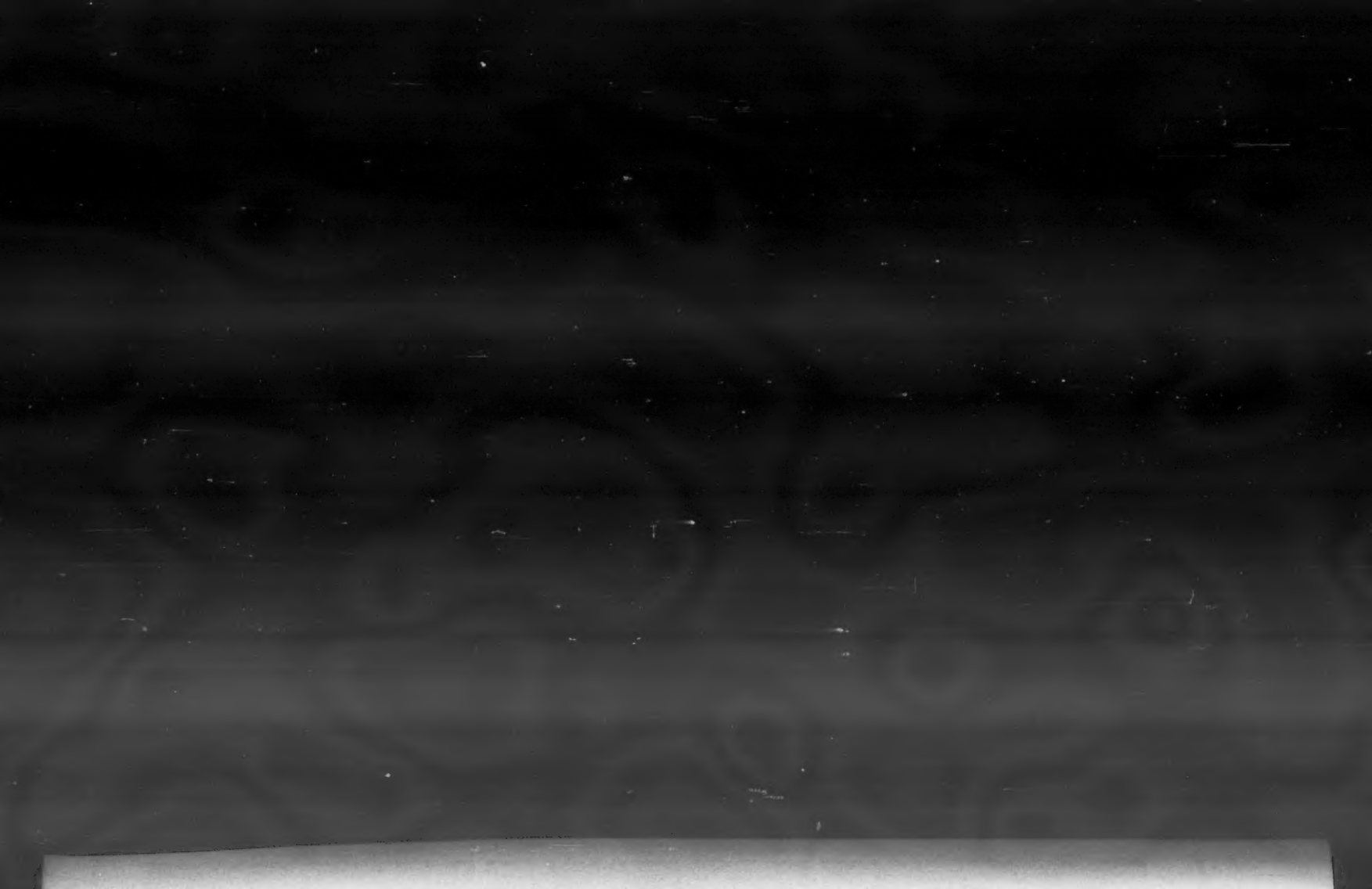
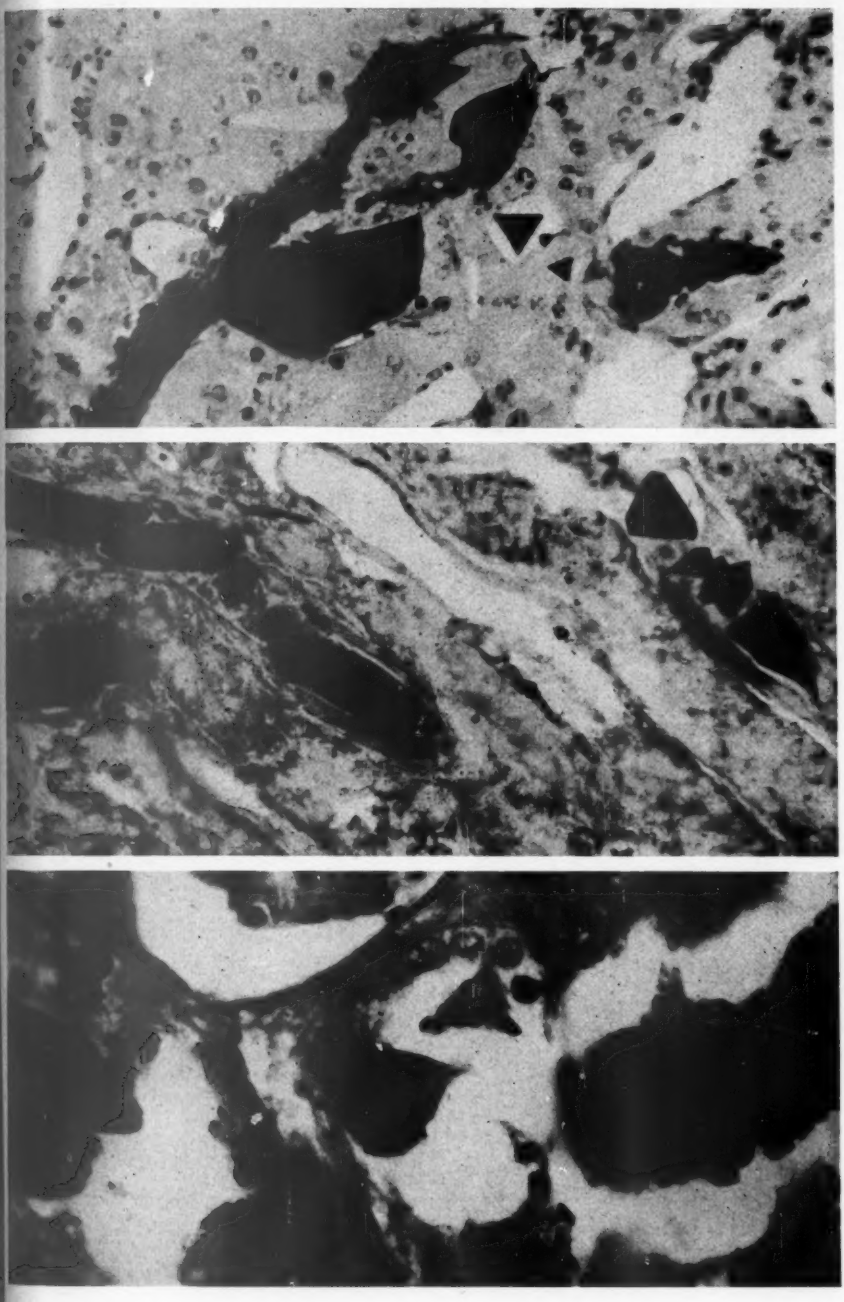
PLATE 144

FIG. 4. Two of the hemoglobin crystals are lying so that the sharp edges are visualized with exceptional clarity. Three large crystalline masses of hemoglobin with angular margins also are seen, one pushed out of the tubular lumen by a wrinkle in the tissue section. × 350.

FIG. 5. Hemoglobin casts, crystals, and globules in kidney of guinea-pig. The globules are out of focus but visible. One crystal is of truncated configuration. × 425.

FIG. 6. The process of phagocytosis of both a large cast and the remaining portion of a crystal is fairly well shown. The nuclei of the cells which have invaded the margins can be seen here; microscopically the cells appear to lie within lacunae. One large triangular shaped macrophage contains hemoglobin globules. The glomerulus shows very little change. × 780.





GLOMERULONEPHRITIS OCCURRING IN EXPERIMENTAL BRUCELLOSIS IN DOGS *

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The production of experimental nephritis has been a problem to which much attention has been given. It has always been difficult to produce, and the results have seldom been consistent with the disease as it is seen in man. Much of the experimental work done in recent years has been directed toward hypersensitivity of renal tissue to some sensitizing agent. Irradiation, high protein diets, and various other forms of injury also have been attempted with varying measures of success.[1-4]

Longcope [5] used horse serum and egg white to produce glomerular lesions in experimental animals, and suggested that nephritis might occur as the result of an allergic reaction.

MacNider [6] thoroughly reviewed the literature up to 1924.

Duval and Hibbard [7] produced acute glomerular lesions in immune and nonimmune rabbits by single large injections of the endotoxic product of the scarlatinal streptococcus, obtained either from the peritoneal lysate of immunized rabbits, or from cultures of the organism treated with activated homologous immune serum. The kidneys in these animals were enlarged, mottled, and showed endothelial proliferation, necrosis, and hyaline thrombi in the glomerular capillaries.

Long and Finner [8] reported the occurrence of proliferative lesions in the endothelial and epithelial elements of glomeruli, with atrophy of the tubules in kidneys of sensitized swine, following the injection of tuberculin into the renal arteries.

Blackman, Brown, and Rake [9] used autolysates of type I pneumococcus to produce lesions in the kidneys of rabbits.

Lukens and Longcope [10] noted the occurrence of hyaline thrombi in the capillary loops with epithelial proliferation in the glomeruli of rabbits in which there was injected into the renal artery a heat-killed vaccine of beta hemolytic streptococcus. Lesions in their animals occurred in 74 per cent of rabbits which had been previously sensitized by intradermal injections of the organism, while lesions occurred in 28 per cent of the nonsensitized rabbits.

Bell and Clawson [11] reported the case of a monkey (*Macacus rhesus*) into which were injected intravenously suspensions of live

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streptococci over a period of 4 years. This animal showed in its kidneys involvement of nearly all glomeruli. The glomerular capillaries were narrowed and occluded by endothelial proliferation and by thickening of the basement membrane of the glomerular loops. There was little fat in the tubules, but atrophy was marked.

VonGlahn and Weld [12] found, in the kidneys of rabbits injected with staphylococcus toxin, congestion, distention of the glomerular capillaries, endothelial necrosis with fibrin thrombi, and changes in the proximal convoluted tubules which varied from cloudy swelling to complete necrosis.

Extensive hemorrhagic cortical necrosis was noted by Rigdon, Joyner, and Ricketts [13] in the kidneys of rabbits injected intravenously with *Staphylococcus aureus* toxin. They found in the less involved kidneys of their animals proliferation of the endothelial lining of the glomerular loops and, in some, hyaline thrombi. The involvement of the proximal convoluted tubules and Henle's loops was extensive, ranging from cloudy swelling to complete cellular disintegration.

More recently investigation of experimental renal lesions has emphasized the nephrotoxic or anti-kidney immune aspect. However, Lindemann [14] had in 1900 produced marked albuminuria, cast formation, and tubular degeneration in rabbits by injecting the serum of guinea-pigs which had been immunized to rabbit kidney. Other experiments along the same line followed.[15] In 1936 Smadel [16] reviewed the literature and reported his work in which anti-kidney serum was prepared by immunizing rabbits with perfused rat kidney and injecting the rabbit serum into rats. He found that specific kidney antibodies, as well as antibodies to other tissues, resulted in lesions or organs other than the kidney.

Horn [17] reviewed the literature up to 1937, covering most of the experimental work done since MacNider's review in 1924. Schwentker and Comploier [18] were the first to prepare a nephrotoxic substance from homologous species. They used both *Staph. aureus* and beta hemolytic streptococcus mixed with rabbit kidney and succeeded in demonstrating antibodies to kidney in rabbits into which this kidney-bacterial mixture was injected. They also demonstrated antibodies to kidney in the blood of 37 of 40 scarlet fever patients tested. They offered the interesting explanation concerning the pathogenesis of glomerulonephritis that the combination of the bacteria or their products with kidney tissue results in some alteration of the renal protein, and that this altered protein constitutes a new antigen as it is released into the circulation during the process of repair. The antibody thus produced is capable of reacting with the altered kidney and possibly with nor-

mal kidney. They thus explain the delay in the human species between infection and the onset of nephritis. Kay [19] explained on this basis the delayed appearance of nephritis in rabbits following injection of the serum of ducks immunized to rabbit kidney.

Cavelti and Cavelti [20-22] also produced glomerular and tubular lesions and demonstrated by serologic methods antibodies to kidney in rats and rabbits injected with homologous kidney and bacterial suspensions.

Simonds and Hepler [23] in recent papers have described the kidneys of dogs following injections of heavy metals (potassium dichromate, mercuric chloride, and uranyl nitrate); snake venoms; diphtheria, staphylococcus, and streptococcus toxins.

The production of specific renal lesions by organisms of the brucella group has not been described in reports on canine brucellosis nor in reports on experimental nephritis. Feldman, Bollman, and Olson,[24] in reporting experiments in brucellosis of dogs, noted in many of their animals the clinical symptoms of disease of the kidney, bladder, and prostate, but were inclined to discount its relation to brucellosis, inasmuch as spontaneous infection in the genitourinary tract is frequent in the dog. At autopsy diffuse cortical scarring was found in the kidneys of these animals. Multiple yellowish nodules in the kidney were described by Thomsen,[25] but no microscopic description is included in his monograph. The kidneys were not found to be involved by others who have described the anatomic lesions of brucellosis in dogs.[26-28]

During the course of experiments performed in this laboratory to determine the effect of organisms of the brucella group on the reticuloendothelial system of dogs and to determine the length of time after infection that the organisms could be recovered from the blood stream, viscera, and lymph nodes, there were found in the kidneys of 4 of 9 animals unusual and interesting lesions. The general histopathologic and bacteriologic observations in these experiments have been reported elsewhere.[29,30] The experimental procedures may be reviewed briefly.

Materials and Methods

Two strains of *Brucella suis* were selected for inoculation into the test animals. Strain A (ABF 36) was isolated from the spleen of a naturally infected hog. It was virulent for guinea-pigs, producing gross lesions and often death in 3 to 4 weeks after intraperitoneal inoculation. Strain B was obtained at autopsy from a case of Hodgkin's disease, and its virulence for guinea-pigs at the time of these experiments was slight. This has been previously referred to as the Brody strain.[31]

Nine healthy dogs were employed. Seven were used for a study of the infection produced by strain B. In 4 of this group the organisms were inoculated intravenously, while in the other 3 the inoculation was intraperitoneal. In the remaining 2 dogs strain A (ABF 36) was inoculated intravenously in one and intraperitoneally in the other. Each injection consisted of 10 billion organisms.

The inoculations were made at intervals of about 1 week, and in some instances they were given in two series, with time allowed for recovery of the animal in order to prolong the experiment, which varied in the individual animals from 186 to 487 days. One animal included here died from ingesting pine shavings on the 38th day.

The dogs were bled frequently from the jugular vein preceding inoculations. Blood cultures, brucella agglutination titers, and opsonocytophagic indices were determined at each bleeding. The details of the bacteriologic observations were fully described previously.[80]

Of the 4 dogs under consideration here, 2 were severely ill at the time of their deaths, on the 198th and 261st day, respectively, of the experiment. In both animals the Brody organism was inoculated intravenously, and inoculations were continued until death in order to produce an overwhelming disease. The first animal (dog 1) received 21 injections, the last injection 14 days before death. Twelve positive blood cultures were obtained from this animal, the last 7 days before death. In the other animal (dog 4) 28 injections were given, with 23 positive blood cultures. The last injection and the last positive blood culture occurred 7 days before death. At autopsy positive cultures of *Br. suis* were obtained from the spleen, liver, and kidney in each animal and from the lymph nodes of dog 1 and the testis of dog 4.

The other 2 animals (dogs 2 and 3) offered a contrast to the first 2 in the degree of clinical illness. Dog 2 received 35 intravenous inoculations of the Brody organism over a period of 37 weeks, during which time it was severely ill. The injections were discontinued and were followed by clinical recovery until the animal was killed on the 487th day of the experiment. Altogether, 18 positive blood cultures were obtained from this animal, the last 233 days before death. At autopsy *Br. suis* was isolated from the lymph nodes and kidney.

In the next animal (dog 3) 39 intraperitoneal injections were given over a period of 45 weeks. The animal was killed on the 461st day of the experiment, 143 days after the last injection. Only 4 of 41 blood cultures were positive, the last 270 days before death. No positive cultures were obtained from the organs at autopsy. During life this dog showed no signs of peritonitis and was not clinically ill at any time.

Results

The general lesions have been described previously, and only the kidneys will be considered here.

In dog 1 focal epithelioid granulomata attributed to the brucella infection were found in the kidney. There were also widespread glomerular lesions, which consisted of extensive fibrosis and hyalinization of the capillary loops with proliferation of the capillary endothelium, and occlusion of some of the glomerular loops. Necrosis was prominent, with an exudate of polymorphonuclear leukocytes and lymphocytes into the basement membrane of many of the fibrotic glomerular loops. In some instances fibrin, albuminous fluid, and cellular débris were found in the glomerular spaces. Adhesions were numerous, and there was usually a definite thickening of Bowman's capsule. Epithelial proliferation was not prominent, but it did occur minimally in many of the glomeruli. In a moderate degree, dilatation and atrophy were found in the tubules.

The lesions found in the kidneys of dog 4 were similar to those just described. They were, however, more acute, with more prominent necrosis and cellular exudation. Hemorrhage into the glomeruli was occasionally found. Cloudy swelling, dilatation, and atrophy of the tubules were prominent. In addition to the changes described there were several linear collections of mononuclear cells, such as are found in spontaneous nephritis in the dog. These appeared to be unrelated to the glomerular lesions.

In the other 2 animals the glomerular lesions were somewhat different from those just described. In dog 2, capillary dilatation with hyaline capillary thrombi and glomerular hemorrhage were found. Endothelial proliferation and increased cellularity of the glomerulus were prominent. Adhesions between glomerular loops and capsule were present but were not numerous. There were many glomeruli which showed a marked diffuse sclerosis, sometimes with thickening of Bowman's capsule, though more often with no capsular change evident. Epithelial proliferation was not a prominent feature. Cloudy swelling in the tubular epithelium was of moderate degree. Vascular changes were not evident. In dog 3 the changes were similar to those just described. No interstitial inflammation was found in either.

Comment

The renal lesions which occurred in these 4 dogs are interesting from the standpoints of experimental nephritis and of chronic brucellosis in the dog. It was demonstrated that *Br. suis* can remain viable over

periods of 5 to 8 months in the tissues of an animal highly refractive to it, and only by repeated massive infection can it produce the disease.

In 2 animals with severe and active disease, active inflammatory lesions were found in the glomeruli. The recent lesions here were superimposed on chronic glomerular scarring, which was obviously not of recent occurrence. In these animals the nature of the experiment was such that one would expect to find a focal embolic glomerulonephritis, such as occurs in bacteremias in man. The lesions here were similar. They probably were the result of repeated sublethal injury to the glomerulus, as is indicated by variations in age and type of the changes in the capillary loops. Some were sclerotic, while others in the same glomerulus were of more recent occurrence with cellular exudation and necrosis. The changes in the tubules were those usually associated with long-standing glomerular injury.

The lesions in the glomeruli of the other 2 dogs are more difficult to interpret. These dogs had had repeated injections early in the course of the experiment; and, although one (dog 2) had been severely ill, it had recovered clinically and had received no injections of the organisms for 8 months prior to death. The other (dog 3) had received 39 intraperitoneal injections, the last injection 5 months prior to death. This animal had not been clinically ill and no organisms were recovered from its tissues at autopsy. The organisms were recovered from lymph nodes and kidneys of the former. There apparently was a progressive renal lesion, histologically more nearly analogous to that of an acute and subacute glomerulonephritis such as is seen in man, and different in structure from the lesions found in the kidneys of the first 2 dogs. It should be emphasized that these lesions differ from the spontaneous interstitial nephritis so common in the dog.[32]

In dog 2 a persistent brucella infection was demonstrated in the kidneys and lymph nodes, while no such infection could be found in the tissues of dog 3. Five of the 9 dogs showed no glomerular lesions. There was no correlation between the level of the agglutination titers to *Br. suis* or the opsonocytophagic index and the occurrence of renal lesions. All titers were high during the entire experiment. Moreover, no correlation could be found between the nephritic lesions and the positive blood cultures obtained in the last 2 animals. Such was not the case with the severely ill and repeatedly infected dogs showing the embolic glomerular lesions. In the last 2 animals (dogs 2 and 3) the mechanism of renal injury is probably that of renal injury of similar type in man, whether it be direct or due to an altered reactivity of the tissues.

There are certain data which would be of interest but which are not

available here. The serologic investigation of antibodies to kidney in the circulating blood during the experiment, and biopsies of the kidneys from time to time should be a part of any future work along these lines.

Summary

1. In experimental brucellosis of dogs there occurred in 4 of 9 animals renal lesions of a type not usually found in dogs.

2. In 2 of the animals necrotizing acute inflammatory lesions involving portions of the glomerulus were found to be superimposed on an older sclerotic glomerulonephritis, such as is seen in focal acute glomerulonephritis in man.

3. In other dogs the lesion was a progressive, active, subacute glomerulonephritis, somewhat similar in structure to the lesion found in man.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

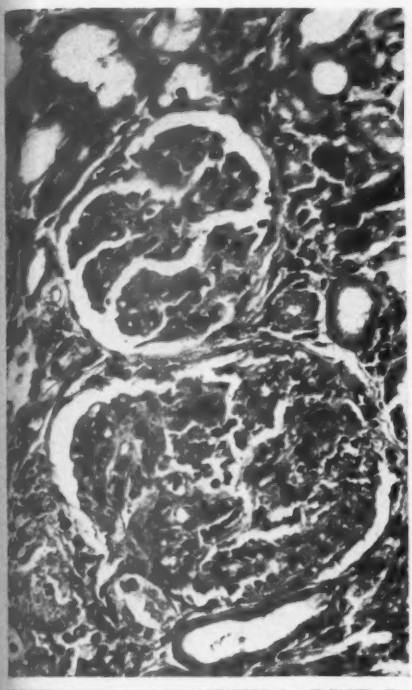
PLATE 145

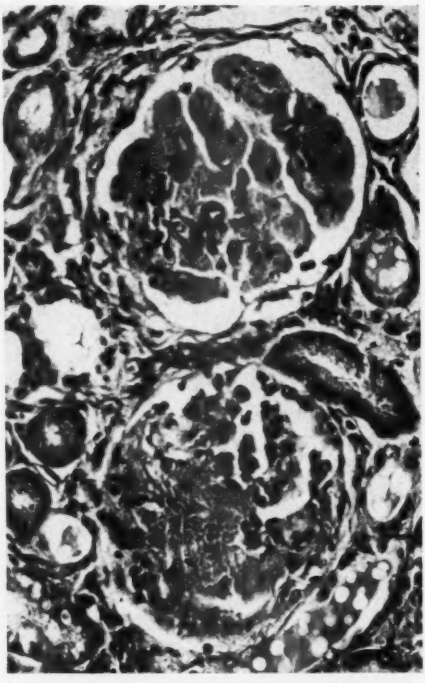
FIG. 1. Dog 1. Extensive acute inflammatory reaction in the lower glomerulus, with scarring and obliteration of the capillary loops, particularly evident in the upper glomerulus. × 265.

FIG. 2. Dog 4. The necrotic lesion with fibrinoid changes, seen in many of the glomeruli of this kidney. × 265.

FIG. 3. Dog 2. Thickening of the capillary basement membrane and endothelial proliferation are characteristic of the changes found in this animal. × 265.

FIG. 4. Dog 3. The changes here are those of endothelial proliferation, scarring, and focal necrosis with glomerular hemorrhage. They are similar to the changes shown in Figure 3. × 325.





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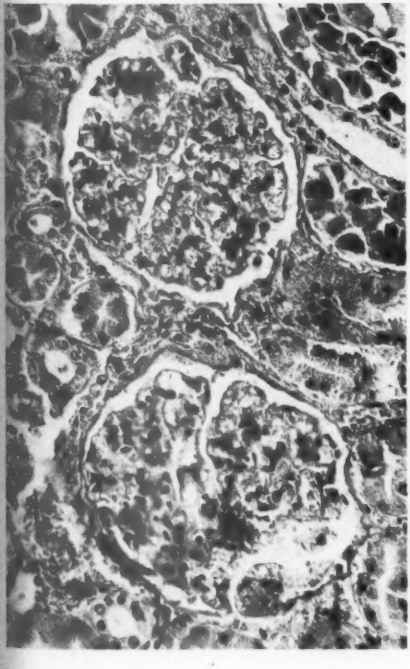

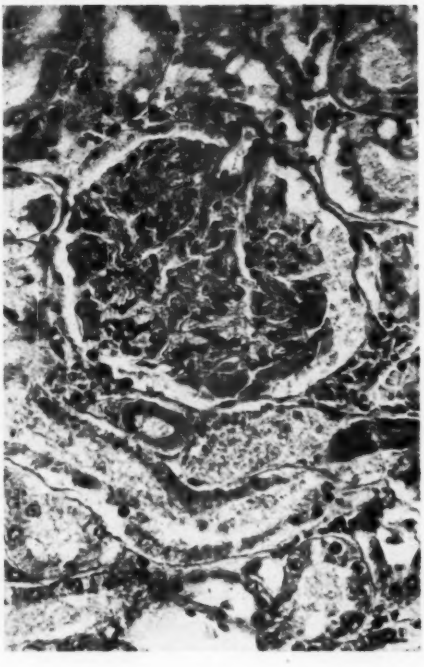

4

FATAL INCLUSION-DISEASE PNEUMONITIS IN AN ADULT*

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While occasional cases of inclusion disease affecting various organs in infants have been reported for many years, the condition remains distinctly rare in adults. The following case represents, so far as I am aware, the ninth to be reported in an adult, and the second example of adult inclusion-disease pneumonitis.

REPORT OF CASE

The patient was a Japanese female, 60 years old, who was admitted to the Royal Victoria Hospital, Montreal, on the service of Dr. J. F. MacIntosh, on June 18, 1945. The admission complaints comprised difficulty in breathing for 7 weeks following a cold, and diarrhea for 7 weeks. The patient's personal history was noncontributory; she had been born in Japan, but had lived in Canada for 32 years. The functional inquiry elicited that about 4 weeks before admission her face had become hot and flushed during the afternoon and her wrists and ankles had become erythematous. Weeping eruptions had occurred at the wrists and ankles without pain or pruritis, but these had regressed somewhat at the time of admission.

On physical examination the temperature was 100° F., pulse, 88; respirations, 35; blood pressure, 96/64 mm. Hg. There was a skin eruption over both wrists and ankles, extending up the forearms and the back of the left leg to the thigh. The lesions were dry, raised, circular, scaling areas measuring up to 6 mm. in diameter. The patient's color was good although there was some respiratory distress. There were decreased vocal fremitus at the posterior right lung base and medium râles at both lung bases.

The patient was subjected to numerous laboratory tests, the majority of which yielded results within normal limits. However, the total proteins of the plasma were found to be 7.65 gm. per cent with a ratio of albumin to globulin of 3.27/4.38. The hemoglobin was 97 per cent with an increased resistance of the red blood cell fragility. There was a leukocytosis of 12,300, with a differential count of 492 staff forms, 6,519 polymorphonuclear cells, 123 mast cells, 4,305 lymphocytes, and 861 monocytes based upon an examination of 100 white blood cells. The corrected sedimentation velocity was 60 mm., and there was a reduced prothrombin concentration and delayed coagulation of the blood. Vitamin assays disclosed a normal amount of riboflavin in the urine and carotene in the blood; however, there was a markedly reduced content of vitamin A in the blood. The Wassermann and Kahn tests were negative. Biopsy of a skin lesion showed slight hypertrophy of the horny layer, some hemorrhage superficial to the stratum granulosum, scattered vesicle formation, slight parakeratosis, and a scanty infiltration of the corium with lymphocytes.

Roentgenograms of the chest taken on three different occasions disclosed a progressing peribronchial disease with some associated interstitial involvement; this appearance then gave place to one of progressive coalescence. A review of these films by Dr. C. B. Peirce, after the diagnosis of the condition was made at autopsy, failed to disclose any pathognomonic roentgenologic features.

The patient was treated with 2,070,000 units of penicillin over a total period of

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17 days without appreciable effect, her temperature remaining at about 100° F. She was affected with increasingly severe dyspnea and cyanosis, and died in respiratory failure 41 days after admission and about 13 weeks after the onset of symptoms.

The clinical impression was of an unusual bronchopneumonia of unknown cause.

POST-MORTEM EXAMINATION

A complete necropsy was performed by Dr. B. A. Levitan 13 hours after death. The only pertinent findings were in the thorax and colon. The heart and pericardial cavity were normal. The pleural cavities contained a minimal amount of clear fluid. The parietal pleurae were smooth. A thin layer of fibrinous exudate covered the visceral pleura of the anterior aspect of the right lower lobe and the posterior and lateral aspects of the left lower lobe. The right lung weighed 800 gm., while the left weighed 1,000 gm. The lungs appeared to be distended and did not collapse. Upon palpation they were firm and noncrepitant except in the right upper lobe where a slight degree of crepitation was elicited. On section the cut surfaces were granular and slightly moist, varying from gray to a very light grayish white. All lobes were involved, the least involvement being in the right upper lobe which was very light grayish white. The larynx, trachea, and bronchi were normal. The peribronchial and mediastinal lymph nodes were not enlarged.

There were multiple intestinal polypi from the transverse colon to the rectum.

No other organs showed significant abnormalities. The salivary glands were not examined.

Bacteriologic Examination. From a swab from the lung a small number of *Streptococcus viridans* and *Escherichia coli* were grown. The heart blood contained some contaminant corynebacteria.

Microscopic Examination

The tissues were fixed in formol-saline solution and selected sections were stained with hematoxylin and eosin, Weigert's fibrin stain, Glynn's stain for bacteria, and Masson's trichrome stain.

The histopathologic findings in the thyroid, parathyroid, heart, liver, spleen, pancreas, jejunum, colon, kidneys, brain, and vertebral bone marrow were not pertinent. Both liver and adrenal glands showed the basophilic cytoplasmic granules described by Santee.[1] The salivary glands and other tissues were not sectioned.

Multiple sections from various parts of both lungs exhibited essentially similar features. The tissue preservation was only moderately good. The pleura was somewhat edematous and hyperemic. Occa-

sionally a minimal deposit of fibrin could be seen on its surface. Bronchi and bronchioles were filled with eosinophilic coagulum, neutrophilic polymorphonuclear cells, and a few large and small mononuclear cells. The epithelium of the bronchi and bronchioles had separated from its basement membrane and could be identified only as small isolated fragments. This epithelium varied from a cuboidal-columnar to a pseudostratified ciliated columnar form. No inclusion bodies were found in the cytoplasm or nuclei of these cells. The lung parenchyma, generally, was greatly altered. The alveoli were distended by large plugs of protein coagulum containing little stainable fibrin, or by masses of inflammatory cells consisting of polymorphonuclear leukocytes with a considerable admixture of plasma cells, large and small mononuclear cells, and swollen macrophages. Among the inflammatory cells, isolated polyhedral septal cells were seen. Many alveoli contained varying proportions of coagulum, fibrin, and inflammatory cells, while extravasated erythrocytes were present in moderate numbers. Atria and bronchioles were filled with similar material. The alveolar walls were widened by edema fluid and their capillary channels were engorged with blood but interstitial inflammatory mononuclear cells were found in relatively few areas. The cells lining the alveoli were large and prominent, varying from a low flattened cuboidal type with a spindle-shaped nucleus to a swollen polyhedral or slightly elongated cell with a round or oval vesicular nucleus. The cells often lost their identity as individual cells, apparently becoming syncytial, and there was a tendency for sheets of cells to separate from the alveolar walls. No definite inclusion bodies were found in these cells; however, they appeared to be transformed into cells that did contain such bodies.

The inclusion-bearing cells varied considerably in size and form, being found attached to the alveolar walls or, often, lying free in the alveolar spaces. They varied in number from one to several per high-power field. The earliest recognizable form was somewhat larger than the cells already described, measuring 14 μ in its major diameter. It usually lay attached to the alveolar wall between two of the swollen cells described above, and bulged further into the lumen than they did. The nucleus became metachromatic or acidophilic and, while not homogeneous, it was more dense and uniform than the vesicular nuclei previously mentioned. Sometimes there was the appearance of many discrete, round, acidophilic bodies and fragmented chromatin within the nucleus. The cytoplasm, too, showed vague, small, basophilic bodies within it and a slightly lacy texture. Cytoplasmic inclusions were not seen in the absence of evidence of nuclear inclusions.

The next stage of development of the inclusion body was more

easily recognized. The affected cells were usually large, being about 18 μ in size. A distinct round or oval inclusion body about 5 μ in length occupied the center of the nucleus. It was usually more acidophilic than metachromatic. Its edges were seldom sharply defined and it often had a honeycomb or conglomerate appearance. Surrounding this body was a halo of nuclear sap with low chromatic power, and surrounding this again was a sharply defined, distended nuclear membrane with a finely divided, marginated chromatin layer. Just within the nuclear membrane several discrete, minute, round or irregular basophilic bodies were seen. The nucleus tended to be somewhat eccentric. The cytoplasm usually contained definite and numerous round or oval basophilic bodies about 2 μ in diameter. These bodies were frequently less densely stained in their central portion than at their periphery. The largest cells seen averaged about 25 μ in size. They were irregular in shape and sometimes contained two or three nuclei that frequently showed different stages in the development of the contained inclusion bodies.

The most advanced forms encountered showed an ovoid body about 8 μ in length. It was homogeneous and markedly acidophilic but occasionally showed a metachromatic core. A very clear halo surrounded it, but due to the size of the inclusion, the achromatic space was reduced to a few μ in width. The nuclear membrane was again distinct and sharp but the number of small basophilic bodies seen within its margin was reduced. The cytoplasm of the largest cells contained as many as 20 or 30 small, round or oval basophilic bodies measuring 1 to 4 μ in diameter and tending to be localized somewhat in one area of the cytoplasm. Occasionally one of these bodies could be seen to indent the nuclear membrane.

Cytoplasmic and intranuclear inclusions similar to those described in the lungs were found in very small numbers in the epithelial cells of the zona reticularis of the adrenal cortex.

Discussion

In 1925 VonGlahn and Pappenheimer [2] reviewed the literature concerning intranuclear inclusion bodies in tissues other than skin and central nervous system. To the 16 previous reports of such lesions in infants they added their own case of intranuclear inclusions in the intestine, liver, and lungs of a 36-year-old male. The description of the pulmonary lesions in their case bears a substantial resemblance to the findings reported here, but the authors did not emphasize cytoplasmic inclusions. The literature was again reviewed by Farber and Wolbach [3] in 1932. They assembled 25 cases, all except that of VonGlahn

and Pappenheimer being of infants. To these they added 26 new cases from a series of 183 consecutive autopsies on infants and children less than 1½ years of age. In these cases the inclusions were confined to the salivary glands in 24, and were found in various of the viscera, including the lungs, in 2. Their description and illustrations of the intranuclear and cytoplasmic inclusions in these 26 cases are identical in all respects with those found in the present case. The authors stated that the inclusion bodies were identical with the virus inclusion bodies of the salivary glands of guinea-pigs as reported by Pearson,[4] while the latter concluded that the viruses of the guinea-pig and of man were "very similar, if not one and the same."

Although inclusion disease of this type is not a rare lesion of the salivary glands, it is distinctly uncommon as a lesion of other epithelial viscera. In the report of a case of widespread inclusion disease in an infant, Kinney,[5] in 1942, stated that only 25 cases had been reported in which organs other than the salivary gland were involved and that in only 11 of these had the inclusions been found in several viscera. It is interesting to note that the disease is apparently quite asymptomatic unless the lungs are affected.

In addition to the adult case with pneumonitis reported by Von-Glahn and Pappenheimer,[2] 7 other examples of inclusion disease in the adult have been found in the literature.[6-10] These included lesions in the stomach and esophagus, and an anal tumor. The inclusions were morphologically identical with those of the present case, with those of the disease in infants, and with those of virus diseases of the salivary glands in guinea-pigs.

Examination of the various reports of both infantile and adult inclusion disease discloses the occasional failure to find cytoplasmic inclusions, and offers positive evidence that inclusions may be found in various organs in cells of both epithelial and mesenchymal derivation. The failure to demonstrate cytoplasmic inclusions does not appear to invalidate the diagnosis of inclusion disease, for the large size of the nuclear inclusions is almost pathognomonic of the lesion. In the present case cytoplasmic inclusions never were found in the absence of evidence of nuclear inclusions. Indeed, if one may reason by analogy, the occasional absence of cytoplasmic inclusions may be expected, for animal experimentation [11] has shown that, in salivary gland disease in guinea-pigs, the formation of intranuclear inclusions precedes the development of cytoplasmic inclusions by 7 to 10 days.

While the lesions of inclusion disease are highly characteristic, there are various other conditions which will give rise to inclusion phenomena with or without giant cell formation. Diseases which give rise to both

cytoplasmic and nuclear inclusions are not numerous, comprising smallpox, paravaccinia, alastrim, Hecht's giant cell pneumonia, and distemper.[12] In all of these diseases the cytoplasmic inclusions are acidophilic and the nuclear inclusions are small. Inclusions that are induced by chemicals such as bismuth or aluminum hydroxide are usually intranuclear, small, and endowed with peculiar staining reactions, but they are occasionally seen in the cell cytoplasm in the same form in which they appear within the nucleus.[13-15] It is obvious that the basophilic cytoplasmic inclusions of inclusion disease are of diagnostic value.

The number of pulmonary diseases in which multinucleated giant cells and inclusion bodies may be found are few, comprising Hecht's giant cell pneumonia, animal distemper, and measles. Hecht's giant cell pneumonia and animal distemper exhibit in the lungs large, irregular, syncytial cells containing many nuclei and showing small acidophilic inclusions in both nuclei and cytoplasm.[12] The giant cells of measles, on the other hand, while approximating the morphologic characteristics of Hecht's giant cells, apparently show acidophilic cytoplasmic inclusions only.[16]

It is apparent that the single or binucleated giant cells of inclusion disease with their complement of very large, acidophilic nuclear inclusions and small, rather uniform, basophilic cytoplasmic inclusions constitute a highly characteristic morphologic entity. That the human disease is actually caused by a virus is not established. However, the general structure of the inclusion bodies is of a type that has been shown to be associated with viral agents in both man and experimental animals. Furthermore, the lesion is morphologically identical with a proved virus disease of the guinea-pig that has been shown to be capable of unusual virulence.[11]

It was indicated in the clinical summary of the present case that the patient suffered from a marked deficiency of vitamin A content of the blood and also developed a skin eruption that might suggest a nutritional deficiency. The coincidence of virus infection and vitamin A deficiency is not a new observation. Among the group of infants showing inclusion disease reported by Farber and Wolbach [3] there were two with the dermatologic manifestations of vitamin A deficiency. Chown [17] maintained that the pathologic changes in the lungs in giant cell pneumonia were a manifestation of vitamin A lack, and Pinkerton *et al.*[12] have pointed out that many of the clinical and pathologic features of distemper resemble those of such a deficiency. While the interpretation of such observations is beyond the scope of a simple morphologic report, it should be pointed out that the patient was given vitamin supplements when her deficiency was discovered, and that no

morphologic evidence of vitamin A privation was found at autopsy. Moreover, there was no apparent mitigation of the patient's clinical condition at any time during her 6 weeks' hospitalization.

Summary

A case of fatal inclusion-disease pneumonitis in an adult is presented with a detailed study of its morphologic features and a comparison of the lesions with those of other relevant diseases that show inclusion phenomena. The coincidence of vitamin A deficiency and some viral diseases is mentioned. This constitutes the second case of inclusion-disease pneumonitis to be reported in an adult.

I am indebted to Drs. H. Pinkerton and Ph. H. Hartz, both of whom have examined sections from the lungs of this case and concur in the diagnosis.

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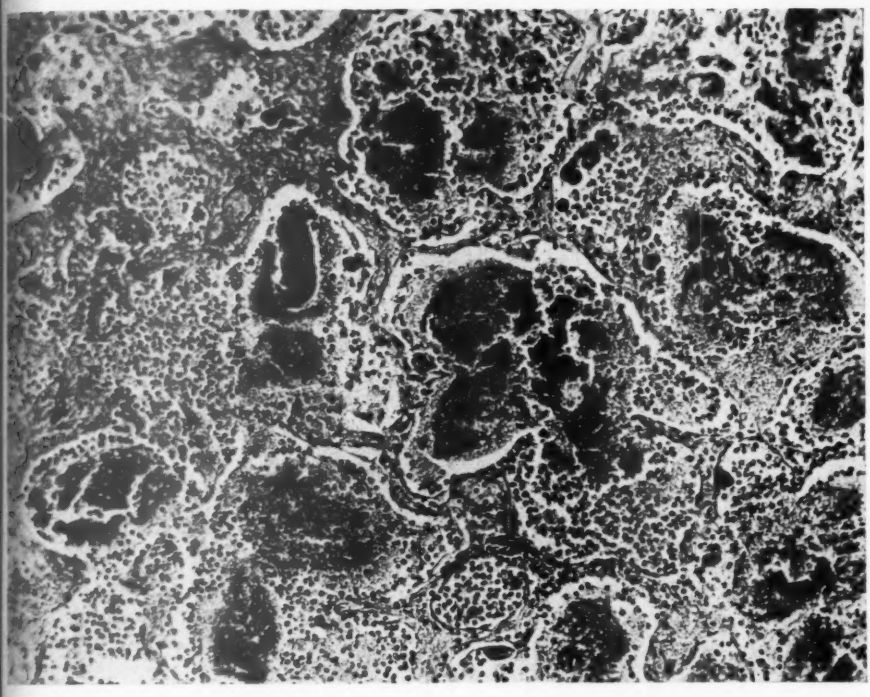
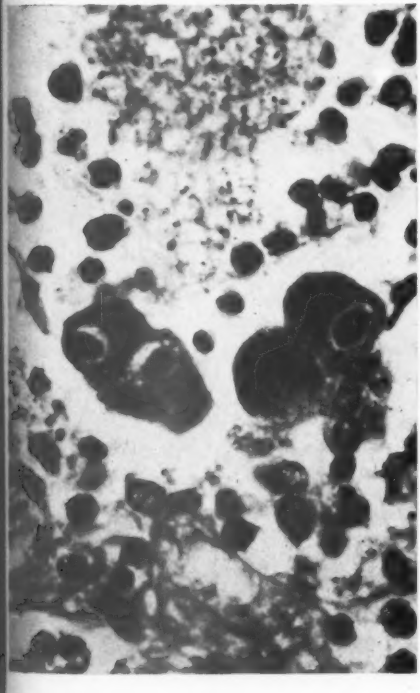
DESCRIPTION OF PLATE

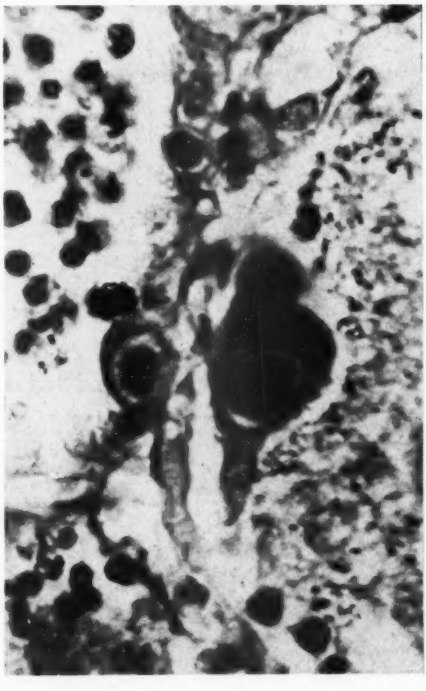
PLATE 146

FIG. 1. Photomicrograph of lung. A low magnification shows hyperemic, swollen alveolar walls enclosing alveolar spaces distended by acidophilic coagulum and inflammatory cells. Numerous inclusion-bearing giant cells lie adjacent to alveolar walls. Masson's trichrome stain. × 130.

FIGS. 2 and 3. Photomicrographs of lung. Detail of the inclusion body giant cells is shown at high magnification. The relation of the cells to the alveolar wall is seen in Figure 2. Of note are the sharply demarcated nuclear membrane, the halo about the intranuclear inclusion, and the large size attained by the latter. Clumped cytoplasmic inclusions are well shown in Figure 2. Hematoxylin and eosin stain. × 860.







3

McMillan Inclusion-Disease Pneumonitis in an Adult

INTRANUCLEAR INCLUSIONS IN THE ISLANDS OF LANGERHANS OF CHICKENS *

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During the course of investigations of cytologic problems in relation to lymphomatosis in chickens, intranuclear inclusions were found in the islands of Langerhans. Since lymphomatosis is the only common disease of chickens at this laboratory and since external and internal parasites are absent except for coccidia in a low percentage of birds,[1,2] there is justification for an attempt to correlate such widely divergent manifestations as inclusion bodies in the pancreatic islets and the occurrence of lymphomatous tumors in various regions of the body. Intranuclear inclusions often indicate the presence of a virus and it has been suggested that filtrates from visceral lymphoid tumors [3,4] and from tumor strains derived by serial passage from naturally occurring lymphomatosis will produce lymphomatous lesions.[5]

The present investigation has been limited to a study of the location and structure of inclusion bodies, the cycle of inclusion formation and degeneration, and the incidence of affected birds in relation to sex, age, and the presence of gross lesions of lymphomatosis.

MATERIALS AND METHODS

The chickens available at this laboratory were Single-Comb White Leghorns. The genetic history and performance of the stock are available in several publications.[1,2,6-8]

Examination was made of all suitably preserved cases from which a section of the pancreas had been saved. This material was from chickens of both sexes and of a wide range in age from 15-day embryos to chickens 1,158 days old. At gross necropsy examination, many birds were negative, some were positive for naturally occurring lymphomatosis, and still more were positive for lymphomatous tumors following inoculation with different lymphomatous tumor strains.

Chickens from 6 different years (1940–1945) were represented, although most of the birds studied were hatched in 1942 to 1944 inclusive. In addition, 10 birds, 161 to 219 days of age, hatched in 1944, were available from the source stock of Iowa State College, which was used in experiments on lymphomatosis.

The fixatives most commonly used were 10 per cent formalin; Zenker's fluid with formalin or acetic acid; and Petrunkevitch's fluids nos.

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1 and 2.[9] Of these fixatives, Petrunkevitch no. 2 was by far the best for showing intranuclear inclusions in the islands of Langerhans. In a few cases fixed in Carnoy's fluid or alcohol-acetic mixture, fair results were obtained. The stains used were Giemsa, MacNeal's tetrachrome, hematoxylin-azur II-eosin, and hematoxylin with eosin or triosin. The hematoxylin and triosin, after Petrunkevitch no. 2, gave excellent results; the inclusions were so brilliantly stained that they were easily identified with the 8 mm. objective. The inclusion bodies were brought out very clearly when material fixed in Zenker-acetic fluid was stained with malachite green and and acridine red.[10] The inclusion bodies are not artifacts of fixation or staining since they could be identified under high magnification with all technics mentioned. However, in many combinations such as formalin fixation followed by hematoxylin and eosin, they appeared pale and often colorless so that they easily could have been overlooked.

The diagnosis of "inclusions present" or "inclusions absent" was based on a single section. The few negative cases found among older birds might have been diagnosed as positive if additional sections had been cut and examined, but it seemed desirable to treat all material in the same way since it would have been impossible to determine positively the absence of inclusion bodies except by complete serial sectioning of every negative case. Every portion of a section was examined under the microscope. In some cases only one inclusion body was found for the entire section; in other cases they were abundant and could be seen instantly. Those slides in which no inclusion body was found were examined thoroughly a second time.

Observations

The intranuclear inclusions were highly tissue-specific in their distribution; they were found only in the islands of Langerhans, never in the pancreatic acini or duct cells, and never in tissues from other organs.

As the examination of slides proceeded, the impression was gained that the inclusion bodies were most commonly found in islets of medium size and that they were usually absent from the extremely large and extremely small islets. Likewise, they were found nearly always in nuclei of cells devoid of granules and rarely in those cells filled with conspicuous, yellow-stained granules. On material fixed in Zenker-acetic fluid and stained with malachite green and acridine red, which in the same slide was discovered to give good tinctorial differentiation of alpha and beta cell granules and also zymogen granules as well as inclusion bodies, it was concluded that the inclusion bodies were

present only in the beta cells of the islets and not in the alpha cells. It was impossible to follow the cell boundaries in the granule-poor cells in material fixed with Petrunkevitch no. 2, and for that reason all boundaries were not included in the drawings. In the alpha cells filled with granules, the cell walls were more definite.

No quantitative measurements were made on the proportion of islet tissue to acinar tissue, and from a survey of the literature it appears not to have been worked out for any avian species beyond the fact that the splenic lobe appears to contain the highest proportion.[11,12] It was observed in the present study that in both the relative amount and in size the islets were small in embryos and young chicks up to several weeks of age. In older chicks there appeared to be a relatively rapid increase both in size of islets and amount of islet tissue. In grown chickens the size of the islets was rather variable, which agrees with Bensley's studies [13] on the guinea-pig. The smallest islet as seen in a single section was composed of a few cells; the largest measured 363 by 458 μ. Cyst-like cavities sometimes were present in the large islets. The cavities were located on the side of the cell opposite the blood vessels. The largest lumen found was 23 μ in diameter. However, in many islets, the lumen, if present, was only a narrow slit. In mammals a lumen among islet cells has been said to be absent [14] and was not reported in the review by Opie,[15] although Clara described a small lumen among the cells of the islands of Langerhans in man [16] as well as in birds.[11] When the lumen was large, the nuclei of the marginal cells sometimes were closely adjacent to the cavity, but if the lumen was narrow and small there was usually no indication of cell polarity as shown by the position of the nucleus.

The acinar portion of the pancreas, in many cases, showed conspicuous lesions of various types. In many birds the acinar tissue was largely depleted of its zymogen granules and the cells and their nuclei were often distorted. Areas in which the cells appeared normally filled with zymogen granules were often multi-focal in distribution. Among the depleted cells many showed a large vacuolated cytoplasm; often the vacuole was large enough to deform the nucleus, and frequently it contained an acidophilic body surrounded by a clear halo. Against the wall of the vacuole was pressed the ergastoplasm or basophilic material of the cell. At first glance the whole configuration gave the suggestion of an intranuclear inclusion, but on closer examination it proved to be entirely cytoplasmic and probably was not an inclusion body of viral type. It was assumed that the acidophilic body inside the vacuole represented an abnormal clumping and degeneration of zymogen granules, or it may be the avian equivalent of Bensley's interpretation [13]

of the Mankowski granules in mammals, but additional data are needed to prove the validity of these suggestions.

In addition to the various cellular changes in the acinar cells, lymphoid accumulations of various sizes frequently occurred. They were usually focal and were located in the interacinar connective tissue or in the loose connective tissue near the larger vessels and ducts. With increased growth of the lymphoid tissue and especially with its extensive growth in tumor strains, both acinar and islet tissues were pushed aside or destroyed. In marked contrast was the complete freedom of the islands of Langerhans from any lymphoid tissue. It was expected that occasionally a few lymphocytes would be found around the small vessels in the islets, as in other regions of the body, but none were observed even when tumors were present in nearby regions of the pancreas.

Description of Inclusion Body and Nuclear Reaction

Typical, resting nuclei (Figs. 1 and 2) were round or oval and showed some variation in size. The chromatin was scattered along the linin fibers and part of it was adherent to the inner surface of the nuclear membrane. No distinct nucleolus was present but some nuclei showed a central clumping of chromatin around a colorless or pale, blue-staining, refractile body (Figs. 1 and 4) which possibly represented an amphinucleolus.

The intranuclear inclusions (Figs. 3 to 20) were strongly acidophilic after fixation with Petrunkevitch's no. 2, and were always homogeneous and never refractile, even when small. There was considerable range in size. Measurements made from the drawings, which had been outlined by means of a camera lucida, gave 0.37 μ for one of the bodies in Figure 4 and 4.15 μ for the large one in Figure 18. Measurements made with a filar micrometer, of cells other than those illustrated, showed a range in size from 0.4 to 4.0 μ, or a 1,000-fold increase in volume from the smaller to the larger.

Early stages in the development of inclusion bodies were considered to be those in which the nuclei showed a minimal amount of retraction of chromatin and linin network toward the membrane. This criterion had proved to be a reliable one in previous studies.[17-20] Initial stages in margination were extremely rare (Figs. 3 and 4). Even in the examples illustrated, margination was well underway and the resulting halo of nucleoplasm around the inclusion body was well developed. In the early stages the inclusion bodies themselves were single or multiple and showed considerable variation in size. Regardless of size, they were always homogeneous and never granular at any stage. Therefore,

it was concluded that these inclusion bodies are of the multiple homogeneous type, and that they are not like the inclusions of panleukopenia in cats,[20] which are granular in the first part of the cycle and become homogeneous in later stages.

In areas where inclusions were relatively abundant, adjacent non-inclusion-bearing nuclei were examined to find earlier stages than those represented in Figures 3 and 4 and to determine whether there might be an indication that the inclusion arose from the plasmosome portion of an amphinucleolus (if the light-staining sphere in the centrally placed clump of basichromatin in Figure 1 can be considered as an amphinucleolus). No transitional stages were found between this plasmosome-like body and the inclusion body. Even more convincing evidence against a plasmosome origin came from the fact that the amphinucleolus-like structure was marginated with the other chromatin (Fig. 4).

There still remained the possibility that the inclusion bodies arose from oxychromatin, but again no transitional stages were observed, although small clumps of oxychromatin were sometimes found in non-inclusion-bearing nuclei. Thus, they were different from the homogeneous inclusions of fox encephalitis in which the acidophilic portion of the inclusion body is derived from oxychromatin.[18]

The next stage after the early reaction was the intermediate phase of the cycle. Practically all of the inclusions in the slides examined were in that phase (Figs. 5 to 18). Some still showed incompletely marginated chromatin and in others all of the chromatin had reached the nuclear membrane. There appeared to be no correlation between size or number of inclusion bodies and the stage of nuclear margination. Therefore, it was impossible to determine whether inclusions (Figs. 5 to 8) might sometimes grow by intussusception of material. However, there was good evidence that they do grow by accretion and coalescence; nuclei such as are shown in Figures 9, 11, 14, 15, and 16 seemed to indicate that the smaller bodies may arrange themselves in the form of a sphere and become merged into a larger body. Sometimes a small sphere was found which lay adjacent to a large one (Figs. 17 and 18) and the form of the inclusion body in Figure 19 seemed to indicate that such a small sphere could merge into a larger one. On the other hand, there seemed to be a certain amount of resistance to fusion of multiple spheres, even when large and touching each other (Fig. 10), and, likewise, when the spheres were small they sometimes showed little inclination to fuse (Figs. 12 and 13) but seemed to be scattered through the nucleoplasm at random.

Late stages in the cycle of nuclear reaction were as hard to find as

the early stages. Only a few nuclei were observed (Fig. 19) in which the marginated chromatin was pulled together in clumps characteristic of karyorrhexis, thus exposing bare, unstained sections of the nuclear membrane. A similar reaction was observed in late stages of fox encephalitis [18] and after subcutaneous inoculations of aluminum oxide.[19]

One nucleus (Fig. 20) was found in which the nuclear membrane appeared to be broken and the inclusion body partly extruded. Rupture of the membrane apparently had caused lysis of some of the chromatin, giving the laked, homogeneous, pale-blue-staining material shown between the nuclear membrane and the inclusion body. (The ring-shaped body near the lower part of the nucleus was considered to be only a circle of chromatin.) It was thought that rupture of the nuclear membrane occurred while the tissue was alive and was not due to the drag of the microtome knife. In the latter case, there would have been no lysis of chromatin. One example is not adequate to determine the final stage in the life of the nucleus, but because the inclusion body seemed to be extruded a search was made for inclusion bodies in the cytoplasm of islet cells. The evidence for the existence of extranuclear inclusions thus far has not been sufficiently definite to be conclusive.

The possibility was tested that the inclusion bodies might contain glycogen since Chipps and Duff [21] reported that empty or ghost nuclei of the liver contained large glycogen spheres which were dissolved in the usual technics. Particularly significant was the fact that 23 per cent of the cases showing glycogen inclusions were from patients with uncontrolled diabetes mellitus. Chicken pancreas was fixed in Carnoy's fluid or in acetic acid-alcohol and stained with Best's carmine. The large inclusions in the islets were negative for glycogen and the small ones sometimes took a faint pink coloration with the stain, which, however, was not sufficiently strong to be regarded as positive for glycogen.

Relation of the Incidence of Inclusion Bodies to Age

The data in Table I show the presence or absence of inclusion bodies of the islets in birds of different ages, the number of each sex involved, and the presence or absence of gross lesions of naturally occurring lymphomatosis or its tumor strains.

Since the cases reported represented a variety of experiments and projects, the distribution of birds at different ages is not uniform; for example, tissues from normal young birds were lacking. Yet, with the

cases available, the results show a definite relationship between age and the presence or absence of inclusions. No inclusion bodies were found in any of 39 birds, from 15-day embryos to chicks 30 days old. Inclusion bodies were present in 230 of 235 birds, or 97.9 per cent, above 30 days of age. When these were broken down into smaller age groups, 75 per cent (6 of 8) were positive at 31 to 40 days of age, 93.7 per cent (15 of 16) were positive at 41 to 50 days of age, 97.7 per cent

TABLE I

Incidence of Intranuclear Inclusions in the Islands of Langerhans in Relation to Lymphomatosis, Age, and Sex

Age in days	No. of cases	Inclusions present				Inclusions absent			
		Lymphomatosis		Negative for lympho-matosis	Total	Lymphomatosis		Negative for lympho matosis	Total
		Tumor strains	Naturally occurring disease			Tumor strains	Naturally occurring disease		
Embryos									
15–21	7							7	7
After hatching									
1–10	12					2	3(?)	7	12
11–20	8					8			8
21–30	12					12			12
31–40	8	6			6	2			2
41–50	16	15			15	1			1
51–100	43	35	6	1	42	1			1
101–300	53	6	8	39	53				
301–500	42		1	41	42				
501–1000	71		6	64	70			1	1
1001–1158	2			2	2				
Totals	274				230				44
Males					68				23
Females					162				18
Sex not determined									3
Totals					230				44

(42 of 43) were positive at 51 to 100 days of age, and 99.4 per cent (167 of 168) were positive at 101 to 1,158 days of age. Thus, the critical age in the transition from the absence of inclusion bodies to their presence was 30 days as based on 10 day age groups.

Ten birds, 161 to 219 days of age (3 males and 7 females), from the source stock of Iowa State College for studies on lymphomatosis, all showed intranuclear inclusions in the islands of Langerhans.

Relation of Inclusion Bodies to Sex

The utilization of a small number of males in breeding pens resulted in a low proportion of males to females in the older birds, but from the

cases available it was found that the presence of inclusions was not associated with one sex more than with the other. As shown in Table I, 23 males and 18 females showed no inclusion bodies, while inclusion bodies were found in 68 males and 162 females.

Relation of Incidence of Inclusion Bodies to Incidence of Lymphomatosis

Those who have studied lymphomatosis usually have not included in their data birds which died before 30 days of age [1] or even before 160 days of age.[22] The 3 cases listed under "naturally occurring disease" in the age group 1 to 10 days (Table I) were questionable cases of neural and visceral lymphomatosis. Birds inoculated with cellular or cell-free filtrates of lymphomatous tumor strains were in the age range of 10 to 171 days, and it was from this material, which was well distributed both before and after 30 days of age, that it was concluded that no relationship exists between the tumor strains and inclusion bodies in the pancreas. The older birds were nearly all from a line which is susceptible to lymphomatosis but due to isolation had shown only a low incidence of the disease. The number of grossly negative birds was large compared with the number of grossly positive birds, but many of the grossly negative birds were microscopically positive. On the basis of gross lesions, there appeared to be no relationship between naturally occurring lymphomatosis and the development of inclusion bodies in the islands of Langerhans. However, no tests have been devised for recognition of the etiologic agent, or agents, of lymphomatosis other than by the presence of the lymphoid tumors. Thus, it is at least theoretically possible that many or even all birds were carrying the disease agent.

The birds listed in Table I were from inbred lines,[1,2] as follows:

Selected for Resistance		*Selected for Susceptibility*	
Line	No. of birds	Line	No. of birds
5	1	2	8
10	1	7	23
		9	2
		11	2
		14	3
		15	231

It is obvious from these data that susceptible line 15 is the only one adequately represented. It might prove to be true later that other lines have a different incidence and age relationship than are shown by the data thus far obtained. The one bird over 500 days of age which was negative for inclusions came from line 2.

Discussion

The data thus far obtained have given the following information: Intranuclear inclusions occurred in the cells of the islands of Langerhans of the White Leghorn chickens studied in nearly all birds over 30 days of age. The structure and location of the inclusion bodies and their cycle of development are in harmony with the idea that a virus might be present. The only disease, except coccidiosis, known to exist in the birds used was lymphomatosis, and there is evidence in the literature [8-5] that visceral lymphoid tumors, both naturally occurring and in a tumor strain, carry a filtrable agent or agents. Yet there was no evidence of correlation between lymphomatosis when based on gross diagnosis and the presence of inclusion bodies in the pancreas.

If the inclusion bodies do signify the presence of a virus, it must be latent, since inclusions are present in some birds which to all appearances are normal and healthy. Andrewes [23] and Oberling [24] have discussed numerous examples of latent viruses. The submaxillary gland virus is also a good example and a review of the literature concerning this virus permits some suggestive analogies with the conditions found in the islets. Surveys have indicated that in one laboratory in England, 32 per cent (6 of 19) of the guinea-pigs were infected with the submaxillary gland virus.[25] In the New York area, 84 per cent of 75 guinea-pigs over 6 months of age carried the virus, and only 3 of 43 under 1 month of age showed inclusion bodies.[26] Thompson [27] found that 14 per cent (10 of 70) of the rats in a laboratory colony were infected and Rector [28] reported that 20 per cent (24 of 120) of wild rats carried inclusions of the submaxillary gland virus. Rector and Rector [29] reported that all of 14 ground moles were infected. Kuttner and Wang [30] observed that nearly all full grown Chinese hamsters carried the submaxillary gland virus. Cowdry and Scott [31] found salivary inclusions in 2 *Cebus fatuellus* monkeys and failed to find them in 18 *Macacus rhesus* monkeys.

There seems to be complete compatibility between the submaxillary gland virus and the host except for slight monocytic infiltrations of the gland. Yet when the infected gland from guinea-pigs is injected intracerebrally into noninfected animals, it causes death in 3 to 16 days.[17,25,26] The natural means of transmission is not known, but Cole and Kuttner [26] found that animals less than 30 days of age were reasonably certain to be free from the disease and this was confirmed by Scott [32] and by Pearson.[33] This is the same age range in which the inclusion bodies are absent in the pancreatic islets of chickens.

The inclusion bodies of the submaxillary gland virus differ in several

ways from those found in the pancreas of chickens. Although both belong to the homogeneous type, the inclusion body of the submaxillary gland virus is usually single and there are never more than two in a nucleus. The nucleus hypertrophies greatly, the chromatin returns from its marginated position on the nuclear membrane to apply itself on the inclusion body and, finally, a cytoplasmic inclusion is present in addition to the one in the nucleus.[17] The inclusions found in the islets are often multiple; there is only slight and often no hypertrophy of the nucleus; chromatin or linin fibers have never been found attached to the inclusion body; and cytoplasmic inclusions are absent. In an examination now under way of pancreases from wild birds, chromatin and linin fibers have been found attached to the inclusion body with a resulting retardation of margination. Multiple intranuclear inclusions of homogeneous type have been reported in the kidney of the Ceropsis goose,[34] and were found in the human kidney after lead poisoning[35] and after subcutaneous injections of aluminum oxide.[19]

In both of the latter examples of inclusions, which represent reactions to metals, considerable chromatin was attached to the inclusion bodies, and in the case of aluminum oxide the inclusion body was shown to arise from the plasmosome portion of an amphinucleolus. In the pancreatic islets of chickens there was no indication of adherent chromatin or of a plasmosome or oxychromatic origin, but since the early stages were few in number, plasmosome origin still remains a possibility.

If the inclusion bodies are not associated with a virus, then it is possible that they have some, as yet unknown, connection with physiologic activities in the islet tissue. An analogous example might be illustrated by the presence of intranuclear crystals in the hepatic cells of the Canidae which, through the work of Weatherford and Trimble,[36] appear to be related to the metabolism of purine bases, particularly uric acid excretion. The Dalmatian showed a lower number of crystal-bearing nuclei per animal than other breeds.[36,37] Crystals were found in 15 of 16 dogs examined. Cowdry and Scott[38] found the crystals in 22 per cent of 68 dogs examined. Weatherford and Trimble[36] concluded that neither age nor sex was a factor in the occurrence of intranuclear crystals. Intranuclear crystalloids have been reported by Dawson[39] in the superficial epidermal cells of the catfish, but no studies as to their cause have yet been made. He suggested that they might be related to degeneration of the cells.

An examination was made of the literature on the growth of the pancreas in birds, on carbohydrate metabolism, blood-sugar levels, and reactions to insulin. The percentile growth curve of the pancreas in

relation to body weight, according to Latimer,[40] showed at first a rise from 0.52 to 0.62 per cent. The latter maximum value was attained when the birds weighed 160 gm. Then followed a gradual fall to 0.20 per cent. The peak at 160 gm., according to Latimer's weight-age curve, fell at 30 days, which from the present study appears to be the critical age in the transition from absence to presence of inclusions.

Only a small amount of data was found in the literature on blood-sugar levels for chickens at different ages. In the embryo [41] it was as high as reported for the adult chicken.[42,43] Heller and Pursell [44] followed blood-sugar levels in hens from 1 to 27 months of age and observed a slight but not significant decrease with age. Batt [45] used chickens 6 months to 4 years of age and stated that "Immature birds tend to have a higher fasting blood-sugar level than adults." Golden and Long [46] found no significant difference in blood-sugar levels of birds weighing less than 300 gm. and those weighing more than 300 gm. In the common pigeon [47] there was a slight rise between the first 33 days and the next period from 57 to 66 days, but Scragglies and Ring Doves showed no differences at approximately the same age periods. Whether pigeons contain intranuclear inclusions in the islands of Langerhans similar to those found in the chicken is not yet known.

A virus disease of pigeons designated as due to an intranuclear inclusion agent has been reported. The intranuclear inclusions are found in both acinar and islet tissues of the pancreas as well as in liver and spleen.[48] This intranuclear inclusion disease was first found associated with psittacosis but was later isolated and the agent proved to be a virus smaller than psittacotic elementary bodies and to have a much greater host specificity.[49] The inclusions of intranuclear inclusion disease are reported to resemble those of herpes. Herpes produces a granular type of inclusion body but in the early stages may show homogeneous masses associated with the granules.[20] Thus it differs greatly from the inclusions found in the pancreatic islets of the chicken, which are homogeneous and multiple.

A report by Jungherr and Lucas [50] on intranuclear inclusions in the spleen of a poult is in process of preparation. Both homogeneous and granular types of inclusions were found but none belonged to the multiple homogeneous type found in the pancreas of the chicken, nor did the inclusions in the two species follow similar cycles of development.

Physiologic conditions as well as viruses can be responsible for intranuclear inclusions. In the survey of literature already presented it has been brought out that the inclusions in the islets of the chicken have certain features in common with either possibility. On the one

hand, the islet inclusions show a similar age relationship to those found in the viral submaxillary gland disease of guinea-pigs and the same high degree of compatibility with the host. In chickens as in guinea-pigs and ground moles there is a high incidence. On the other hand, a purely physiologic relationship, as seems to be the case in the crystals found in hepatic nuclei of dogs, is at present only a theoretical possibility in chickens because the physiologic data which might lead to correlations between structure and function are lacking. However, like dogs, chickens show a high incidence of inclusions in the populations studied and in both species there are no apparent pathologic lesions directly associated with the inclusion bodies.

It is evident, therefore, from the information and data thus far available that there is needed a study of the pancreas from numerous breeds of chickens and from various sources, as well as knowledge of the presence of similar inclusion bodies in species of birds which apparently do not carry the agent of lymphomatosis. Transmission studies are needed to determine whether a virus is present. The questions remain, also: Is the presence of intranuclear inclusions damaging to the normal function of the islands of Langerhans, and is the normal inter-relationship of the islets to hormone actions of adrenal, pituitary, and sex endocrines adversely affected?

Summary

1. Intranuclear inclusions of the multiple homogeneous type were found in the islands of Langerhans of the White Leghorn chicken.

2. The cycle of development and degeneration of the inclusions was followed. The number of inclusions found within a nucleus varied from one to over a dozen. They ranged in size from 0.4 to 4.0 μ. Growth took place in some cases by coalescence of small inclusions to form larger ones, and growth by intussusception might have occurred also. In still other cases they remained multiple in character. Early stages were rare; in most nuclei, margination had proceeded far enough to give a well defined halo. Chromatin or linin fibers were never found attached to the inclusion body. Cytoplasmic inclusions were absent.

3. The presence of these intranuclear inclusions was related to the age of the bird. None was present under 30 days of age. In chickens 31 to 40 days of age they were present in 75 per cent of the birds; from 41 to 50 days of age, 93.7 per cent were positive; from 51 to 100 days of age, 97.7 per cent were positive; and from 101 to 1,158 days of age, 99.4 per cent contained inclusion bodies.

4. Sex seemed to have no influence on the incidence of the inclusions.

5. There was no correlation between the presence of inclusion bodies in the pancreatic islets and lymphomatous lesions as identified by gross examination only.

6. No definite significance of the inclusions in respect to latent virus diseases, growth, and physiologic factors can be assigned at the present time.

Since completion of the manuscript it has been brought to my attention that similar intranuclear inclusions of pancreatic islets have been observed by Jungherr and Levine.[51] In their study of the pathology of pullet disease they suggested that "Pathologically the condition is characterized by . . . . enlargement and apparent injury of the pancreatic islands." Other lesions associated with pullet disease were included besides the one mentioned. It must be concluded from studies on chickens at this laboratory, in which there has never developed a case of pullet disease, that the intranuclear inclusions of the pancreatic islets have no association with this disease.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

Drawings were made at an optical magnification of 1800 × and traced with a camera lucida at a projected magnification of 4076 ×.

PLATE 147

All nuclei are from beta cells of the islands of Langerhans. The tissues were fixed in Petrunkevitch no. 2 and stained with hematoxylin and triosin.

FIG. 1. A normal, noninclusion-bearing nucleus. The central, dark-stained chromatin mass enclosing a lighter stained sphere may represent an amphinucleolus.

FIG. 2. A normal noninclusion-bearing nucleus with diffusely scattered chromatin.

FIGS. 3 and 4. Early stages in the formation of inclusion bodies.

FIG. 3. The diffuse chromatin network beginning to marginate.

FIG. 4. Two inclusions, each measuring 0.37 μ in diameter. The amphinucleolus-like structure marginates with the chromatin and takes no part in the production of the inclusion body.

FIGS. 5 to 18. Intermediate stages of nuclear reaction.

FIGS. 5 to 7. Nuclei in which the chromatin has not fully marginated but the inclusions have grown to considerable size.

FIGS. 8 and 9. Similar stage of nuclear reaction as shown in Figures 5 to 7, but the inclusion bodies are multiple.

FIG. 10. A completely marginated nucleus which contains three large inclusions of equal size.

FIG. 11. A cluster of inclusion bodies in a partly marginated nucleus.

FIGS. 12 and 13. Multiple inclusions scattered at random with no tendency to aggregate in the form of a hollow sphere.

FIGS. 14 and 15. Multiple inclusions arranged in the form of a sphere. It is not certain whether the central homogeneous area in Figure 15 represents a larger inclusion body or whether it is merely the optical effect of the peripheral bodies at higher and lower levels.

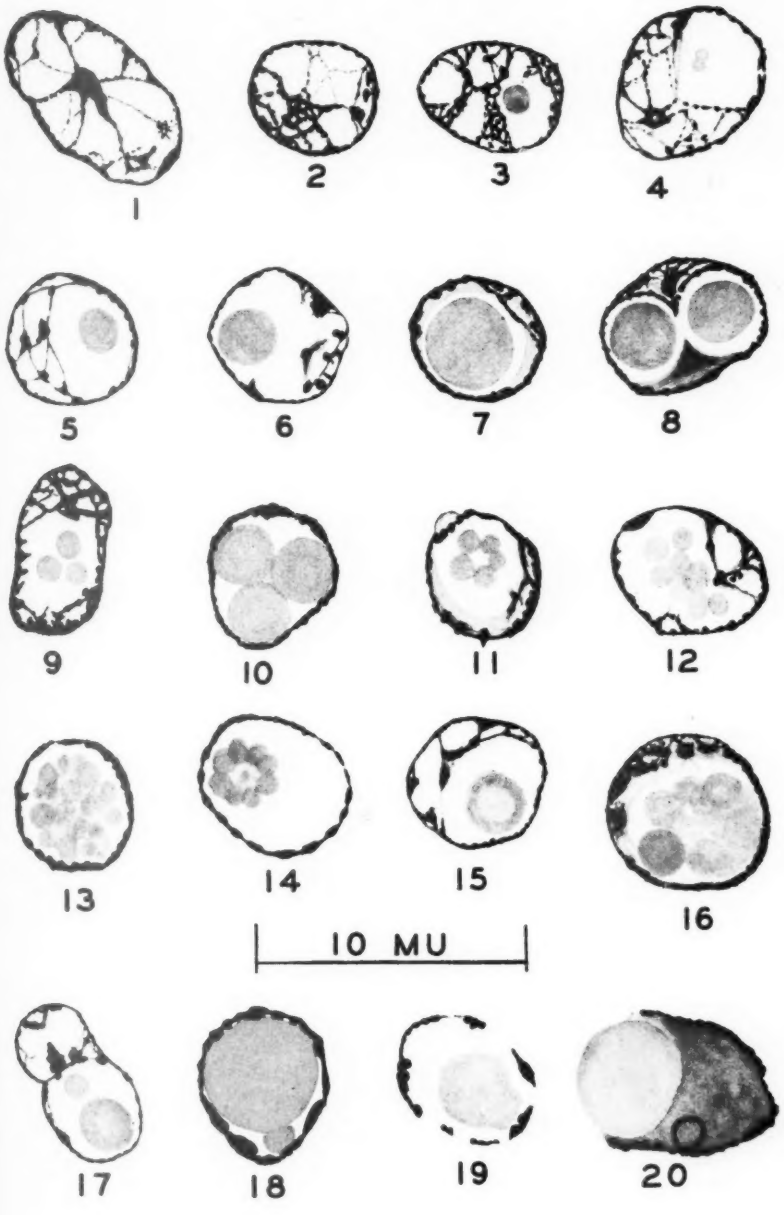
FIG. 16. Peripherally placed inclusions fusing with the central inclusion mass.

FIG. 17. A lobulation of the nucleus somewhat like Figure 8. This nucleus contains two additional inclusions not shown, one at a low level of focus and one in the upper nuclear lobe.

FIG. 18. Extreme inequality of size of inclusion bodies in the same nucleus. The larger of the two measures 4.15 μ in diameter.

FIG. 19. A late stage in nuclear reaction in that the marginated chromatin is pulling together in clumps (karyorrhexis) leaving bare the unstained nuclear membrane. From the shape of the inclusion body it seems evident that a smaller inclusion is fusing with a larger one.

FIG. 20. A late stage showing a rupture of the nuclear membrane with liberation of the inclusion and laking (karyolysis) of the chromatin.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THE FATE OF ENDOCARDIAL VEGETATIONS FOLLOWING PENICILLIN TREATMENT OF BACTERIAL ENDOCARDITIS *

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The apparent curability of both acute and subacute bacterial endocarditis with penicillin has recently become well established clinically. Several clinics, including our own, have reported groups of cases in which such treatment has been followed by the disappearance of all symptoms and signs of the infection and sterilization of the blood for periods now reaching 3½ years. Such enduring remissions in a disease that usually runs its course to death within 1 year speak convincingly of cure. The argument that these cases may not be healed and merely represent examples of the rare or hypothetical "bacteria-free" stage of the disease is emphatically refuted by the considerable number of clinically cured cases already reported within the short period of 2 years. However, the complete and ultimate proof of cure rests on the demonstration of unequivocally healed and sterile remnants of vegetations in previously treated cases following death from other causes. This report presents four such cases.

PRESENTATION OF CASES

Case 1

A. G., a man, 60 years old, developed shaking chills and protracted fever as a probable complication of thrombophlebitis following a recent saphenous vein ligation for varicose veins and ulcer. Blood cultures repeatedly yielded a heavy growth (about 500 colonies per cc.) of *Staphylococcus aureus* (coagulase +).

On admission on February 7, 1944, he was gravely ill and in a stupor. Many petechiae were present on the conjunctivae and extremities. A harsh systolic aortic murmur, audible in the neck and arms, was present without thrill. After the first week the auscultatory and physical signs of aortic insufficiency developed. Penicillin was given intramuscularly at intervals of 2 to 4 hours, in doses of 20,000 units for the first 3 days, and in 10,000 unit doses for 18 days longer; the total given was 1,940,000 units. Fever and petechiae disappeared at the end of the first week and did not return. All of 8 blood cultures after the inception of treatment were sterile. However, toward the end of the second week of treatment attacks of acute paroxysmal dyspnea and pulmonary edema appeared, and progressive and intractable congestive failure progressed in spite of the usual therapeutic efforts to control heart failure with digitalis, oxygen, salt restriction, and diuretic drugs. The patient died on the 21st hospital day in acute pulmonary edema.

Autopsy was performed 5 hours after death by Dr. A. Newman. The significant findings follow.

Gross Examination. The heart weighed 515 gm. and all chambers

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were dilated. The right ventricle measured 5 mm. in thickness; the left, 12 mm. The circumferences of the tricuspid, pulmonic, mitral, and aortic valve rings were 13, 7, 9, and 7 cm., respectively. Irregular fibrous and calcified elevations were present on the tricuspid and mitral leaflets along the line of closure. The chordae tendineae of the mitral valve were thickened and shortened and a few were fused and inserted into the thickened free edge of the leaflets. The aortic valve cusps were thickened, calcified, and fused; the right posterior cusp was swollen and red, and the aortic surface of the cusp was covered by a red-brown, friable thrombus. A small aneurysm was present in the posterior wall of the aorta above the valve ring. The mouth of the aneurysm was 4 mm. in diameter and the structure measured 6 mm. in diameter. A zone of exudate was seen about the aneurysm in the soft tissue at the base of the heart.

The lungs weighed 630 and 690 gm., and yielded foamy fluid when cut; there were no infarcts. The spleen was soft and friable and weighed 370 gm. The right and left kidneys weighed 190 and 218 gm., respectively. The cut section revealed a swollen cortex and indistinct tubular markings. The liver, which weighed 1475 gm., was brown and the lobular markings were accentuated.

Microscopic Examination. On microscopic examination the myocardium was well preserved and the cells hypertrophied. The leaflets of the mitral valve were thickened by hyalinized connective tissue and were devoid of cellular exudate. Section of the right posterior cusp of the aortic valve showed masses of bright eosinophilic material within the cusp undergoing organization and calcification (Fig. 1). Fresh and organizing thrombi were present on the endocardial surface over these lesions. No organisms were detectable with special bacterial stains. The aneurysm in the aorta was lined by thrombus and the wall was composed of granulation tissue containing numerous polymorphonuclear leukocytes. This exudate extended into the peri-aortic tissues. No bacteria could be demonstrated in the wall of the aneurysm, in the surrounding tissues or in the thrombus in the lumen by the Brown-Brenn Gram stain.[1] Small zones of fibrinopurulent pneumonia were present in the tissues about the bronchi. The lungs, liver, and spleen showed acute and chronic passive congestion. Rare adhesions between the glomerular tuft and Bowman's capsule were found in the kidney, but no other lesions were present to indicate focal embolic nephritis.

Bacteriologic Examination. Post-mortem blood cultures were sterile, as were cultures of the aortic valve. Splenic culture yielded *Bacillus coli.*

Anatomic Diagnoses. Organizing vegetation of aortic valve; mycotic

aneurysm of ascending aorta; cardiac dilatation and hypertrophy; chronic rheumatic mitral valvulitis; acute and chronic passive congestion of viscera; hydrothorax (bilateral); focal pneumonia. (*Staphylococcus aureus* septicemia present 3 weeks before death; diabetes mellitus.)

Death in this case resulted from intractable congestive cardiac failure.

Case 2

T. J., a woman, 22 years old, had had chorea 11 years before her fatal illness, followed by acute nephritis 1 year later. Three days before admission she became acutely ill with fever (104° F.), epistaxis, sweats, and headache. During the next 2 days pain developed in the left foot and wrist, and an Osler's node appeared on the left thumb.

She was acutely ill with high fever, tachycardia, and a blood pressure of 80/50 mm. Hg. About 12 petechiae were found in the skin, under one eyelid, and under one fingernail. The left wrist was swollen and tender. The moderately enlarged heart presented a fairly loud and slightly coarse systolic murmur at the apex. Laboratory examinations disclosed a moderate anemia, a relative polymorphonuclear leukocytosis, microscopic hematuria, and a few granular casts in the urinary sediment. Blood culture was heavily positive for hemolytic *Staph. aureus* (coagulase +, mannite +). The admission diagnosis was rheumatic heart disease with mitral valvulitis and insufficiency, and acute bacterial endocarditis probably on the mitral valve.

Treatment consisted of immediate transfusion and institution of massive penicillin therapy by continuous intravenous drip. The dosage of penicillin began at 240,000 units per day but was soon increased to 500,000 and then to 1,000,000 units per day when it was found that the infecting organism was relatively resistant to penicillin (0.05 units per cc. were required to inhibit growth *in vitro*), and that smaller daily doses failed to yield serum penicillin concentrations above 0.16 units per cc. of serum (due, probably, to a high penicillin "K" content of the product). Treatment was discontinued after 31 days during which 18,520,000 units of penicillin had been administered.

Although the patient improved symptomatically, and bacteremia and embolic phenomena disappeared early in the course of treatment and remained absent for more than 1 month thereafter, fever of moderate grade persisted. During the last 3 weeks of life she again became seriously ill with symptoms and signs of pleuritis and pneumonitis (no pathogenic bacteria could be recovered from the sputum). These manifestations were progressive. Dyspnea, cyanosis, and edema developed in spite of the administration of digitalis, and the patient died on the 70th hospital day. Seventeen successive blood cultures in the course of 5 weeks following the termination of penicillin treatment had been sterile, but the last blood culture, 3 days before death, yielded a scant growth of hemolytic *Staph. aureus*.

Autopsy performed 11 hours after death by Dr. B. Lown revealed the following significant findings:

Gross Examination. The heart weighed 310 gm., and only the left auricle was dilated. The right ventricle measured 4 mm.; the left, 12 mm. in thickness. The tricuspid and pulmonic valves measured 11.5 cm. and 6.5 cm., respectively, and showed no changes. The chordae tendineae of the mitral valve were thickened, pale, and opaque,

and the fused cords inserted upon the rounded and thickened free edges of the leaflets. A small, flat vegetation, measuring 4 by 7 mm., was found on the anterior leaflet of the valve. On the posterior leaflet was a hard, white, raised, glistening mass measuring 1.5 by 2 by 1 cm. This vegetation occluded a large portion of the valve orifice, which measured 6.7 cm. in circumference (Fig. 2). The aortic valve was 5.5 cm. in circumference and was composed of delicate cusps with no fusion of the commissures.

The lungs each weighed 700 gm. Both upper and lower lobes were dark red and were firm and dry when cut. The right middle lobe and the bronchi exuded frothy fluid. The vessels were free of occlusions. The spleen weighed 175 gm. and was not remarkable except for a large infarct in the central portion. The liver weighed 1435 gm. and the lobular architecture was accentuated. The right kidney weighed 180 gm. and was congested; the left, 75 gm. A large, irregular, opaque, gray-white infarct occupied a major portion of this kidney.

Microscopic Examination. On microscopic examination the myocardial cells were well preserved and not hypertrophic. Throughout the interstitial tissue were numerous Aschoff bodies composed of large mononuclear cells with characteristic owl-eyed nuclei, multinucleated giant cells, and eosinophilic collagen. The vegetations on the mitral valve consisted of endothelium-covered foci of eosinophilic material, surrounded by granulation tissue and hyalinized fibrous connective tissue (Fig. 3). No organisms were seen in bacterial stains of these lesions.

The alveolar walls in the diffuse areas of pneumonia were thickened by edema fluid and polymorphonuclear and mononuclear leukocytes. An eosinophilic membrane lined the alveoli and respiratory bronchioles. Within the alveoli were mononuclear cells containing blood pigment, occasional polymorphonuclear leukocytes, and numerous red blood cells. Occasional hyaline thrombi were noted in capillaries and arterioles. Organizing infarcts were present in the spleen and left kidney.

Bacteriologic Examination. Cultures of the vegetation on the mitral valve yielded no growth. Culture of the right lower pulmonary lobe yielded hemolytic *Staph. aureus* (coagulase +, mannite +).

Anatomic Diagnoses. Active rheumatic myocarditis; healed rheumatic mitral valvulitis; healing vegetative endocarditis of mitral valve; infarcts of spleen and left kidney; congestion of viscera; hemorrhagic and interstitial pneumonia with capillary thrombi (compatible with rheumatic pneumonia).

Death evidently was due to active rheumatic myocarditis, pneumonia, and congestive failure of the heart.

Case 3

J. F., a female, 64 years old, had been ill for 4 days with fever and stupor that failed to respond to sulfonamide drugs, whereupon she was admitted to the hospital on January 18, 1944. Except for asymptomatic hypertension of at least 3 years' duration, she denied illness in the past. The patient was comatose, had fever of 103° F., and tachycardia of 140 per minute. There were signs of meningeal irritation, basal pulmonary edema, purulent conjunctivitis, and a small draining pustule at the base of the left great toe. Petechiae were noted on the eyelids. The spinal fluid was sterile but contained 24 polymorphonuclear leukocytes per cmm., and the protein content was 97.6 mg. per cent. Two successive blood cultures yielded *Staph. aureus* (coagulase +). The same organism was grown from the conjunctival exudate, the pustule on the toe, and from urine which also contained considerable albumin and pus.

Penicillin was begun on the third day in doses of 10,000 units every 2 hours intramuscularly for 3 days, when the doses were doubled for 6 days, and then reduced again to 10,000 units every 2 hours for 5 days longer. A total of 1,390,000 units was given in 15 days. Although petechiae disappeared and the blood remained sterile after the second day of treatment, the patient continued moderately febrile and acutely ill with renal failure characterized by oliguria, azotemia, and the electrolyte pattern of acidosis. Signs of congestive failure were controlled with digitalis. Although the spinal fluid cleared, convulsions developed and the patient unexpectedly was found dead 5 days after penicillin treatment had been terminated.

Autopsy performed 4 hours after death by Dr. H. Arnold revealed the following findings:

Gross Examination. The heart weighed 380 gm. The left auricle was dilated and lined by thick, white, wrinkled endocardium. The mitral orifice was reduced to a crescentic slit, 1.5 cm. in length, surrounded by cartilaginous opaque leaflets 3 mm. in thickness. On the auricular surface of the anterior leaflet, 2 mm. from the line of closure, was a friable and soft gray-brown vegetation 0.75 cm. in diameter. A small hemorrhage was present in the anterior leaflet near the line of closure. The chordae tendineae were thick, short, and fused, and they inserted into the rounded free edge of the valve. The tips of the papillary muscles were scarred. The endocardium of the enlarged and hypertrophied left ventricle was transparent. The aortic valve cusps were stiff and inelastic but were not fused. No gross changes were present in the right chambers of the heart.

The spleen weighed 140 gm. and was soft and friable. The kidneys each weighed 240 gm. The decapsulated surfaces were finely granular and mottled yellow-brown. Numerous small abscesses were scattered throughout cortex and medulla. The parenchyma was swollen and had a parboiled appearance. The left lobe of the liver contained several encapsulated abscesses less than 1 cm. in diameter. The brain contained an abscess measuring 1.5 by 1 cm. in the left frontal lobe and a smaller abscess in the left occipital lobe.

Microscopic Examination. Brown-Brenn Gram stains [1] of the or-

ganizing mass of fibrin, red blood cells, and polymorphonuclear leukocytes on the auricular surface of the fibrotic mitral leaflet revealed no organisms (Figs. 4 and 5). Gram-positive cocci were demonstrated in abscesses of liver, kidneys, and brain. In addition, lesions of focal embolic nephritis were present in the kidneys.

Bacteriologic Examination. Culture of the blood at necropsy yielded no growth. *Staph. aureus* was grown from the liver abscess.

Anatomic Diagnoses. Healed rheumatic endocarditis with mitral stenosis; healing vegetative mitral endocarditis (vegetations free of organisms); abscesses in liver, kidneys, and brain; focal embolic nephritis; edema; decubital ulcers.

Death in this case seemed attributable to cerebral and renal abscesses and renal failure.

Case 4 *

R. B. was a female, 18 years old, with known mitral stenosis. In May, 1944, she developed subacute bacterial endocarditis due to *Streptococcus viridans.* She was treated with 12,000,000 units of penicillin given in 54 days, largely by continuous intravenous drip. From December 9, 1944, to June 14, 1945, she was ambulatory, entirely asymptomatic and afebrile, and all of 10 blood cultures in this interval were sterile. Two weeks following the extraction of an abscessed tooth on June 14, 1945, slight fever and malaise reappeared and cultures of the blood were again heavily positive for *Str. viridans.* Immediate treatment with penicillin by continuous intravenous infusion of 240,000 units daily for 22 days again brought about apparent clinical and bacteriologic cure. Late in August, symptoms and signs of cerebral and meningeal infection suddenly developed, and cultures of the blood were persistently positive for *Candida albicans.* Therapy with penicillin in massive doses for 3 weeks, and later sulfadiazine and potassium iodide, proved ineffectual. Death occurred on October 5, 1945.

An autopsy was performed 3 hours after death by Dr. H. D. Axilrod.

Summary of Anatomic Diagnoses. Healed mitral and aortic endocarditis; mitral stenosis; myocardial fibrosis; cardiac hypertrophy and dilatation; chronic passive congestion of viscera; healed vegetative endocarditis, Figure 6 (residuum of previously cured *Streptococcus viridans* endocarditis); healed infarcts of spleen and kidneys; acute vegetative endocarditis involving chordae tendineae of mitral valve and left auricular endocardium (*Candida albicans*); rupture of chordae tendineae; acute and organizing focal myocardial necrosis; fresh infarcts in spleen, kidneys, and right basal ganglia; focal embolic glomerulonephritis; petechiae in left conjunctiva; multiple granulomata of brain; ruptured mycotic aneurysm of left middle cerebral artery; subarachnoid hemorrhage; granulomatous leptomeningitis (*Candida albi-*

* This case has been reported in detail elsewhere (Geiger, A. J., Wenner, H. A., Axilrod, H. D., and Durlacher, S. H. Mycotic endocarditis and meningitis; report of case due to *Monilia albicans. Yale J. Biol. & Med.*, 1946, 18, 259–268.

cans); acute tracheitis and bronchitis; acute and organizing focal pneumonia, bilateral.

Bacteriologic Examination. Candida albicans was recovered from the lungs, spleen, kidneys, brain, liver, blood, and the vegetation on the mitral valve.

Death obviously resulted from overwhelming systemic dissemination of infection with *Candida albicans*.

Comment

The four cases herein described constitute anatomic proof that the infective component of bacterial endocarditis is eradicable with penicillin. Such pathologic observations, together with the favorable clinical experiences of the past few years, complete the chain of evidence that bacterial endocarditis is curable.

The nature of the healing process in its several stages was well seen in histologic study of the vegetations. A mass of granulation tissue and hyalinized connective tissue, surrounding eosinophilic material and covered by endothelium (case 2, Fig. 3), apparently represented an early phase. Also obviously early was the organizing mass of fibrin, erythrocytes, and leukocytes that composed the vegetation in another instance (case 3, Figs. 4 and 5). More advanced organization and the deposition of calcium salts within the vegetation appeared to indicate a later stage (case 1, Fig. 1). Finally, the completely healed lesion was exhibited by a pale, hard, and smoothly endothelialized mass of dense connective tissue and areas of calcification (case 4, Fig. 6). It is noteworthy that bacteria were neither seen in, nor cultivated from, the depths of any of these lesions, whether healing or healed, proving that vegetations actually become free of bacteria. Furthermore, the character of the healing or healed lesion in these cases resembles that described by Rosenblatt and Loewe [2] in two cases that had been treated with penicillin and heparin. The absence of thrombotic accretions on the vegetations implies that anticoagulant medication is not an essential adjunct of therapy.

Focal embolic nephritis was not evident in cases 1 and 2, but was present in cases 3 and 4; the last was an instance of active mycotic infection with endocarditis and systemic dissemination at death.

The fatal outcome in case 1 resulted from congestive failure of the heart which developed abruptly and progressed intractably and rapidly in spite of all therapeutic efforts. This result was probably related to the aortic valvular location of the destructive bacterial endocarditic process, with the consequent sudden imposition of undue strain upon a left ventricular muscle that had insufficient opportunity to compensate

through hypertrophy. It is interesting that the two cases of healed bacterial endocarditis studied post-mortem and reported by Rosenblatt and Loewe [2] also showed serious destruction of the aortic valves and both patients had died of congestive failure that progressed relentlessly. There are two obvious implications in this observation: (1) In cases of bacterial endocarditis involving the aortic valve, it is essential that effective antibacterial treatment be instituted at the earliest opportunity, particularly in the presence of acute endocardial infection with rapidly destructive organisms such as the staphylococcus; (2) Convalescence in patients with vegetations on the aortic valve should be prolonged, and return to physical activity should be undertaken slowly, under careful observation, and with skillful institution of the usual measures employed in the control of congestive failure.

Summary

Four cases of bacterial endocarditis, treated with penicillin, presented both histologic and bacteriologic evidence of cure. The healing process evidently results in the disappearance of bacteria from the lesions, not merely their incarceration within the vegetations.

REFERENCES

1. Brown, J. H., and Brenn, L. A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections. *Bull. Johns Hopkins Hosp.*, 1931, **48**, 69–73.
2. Rosenblatt, P., and Loewe, L. Healed subacute bacterial endocarditis. *Arch. Int. Med.*, 1945, **76**, 1–10.

DESCRIPTION OF PLATES

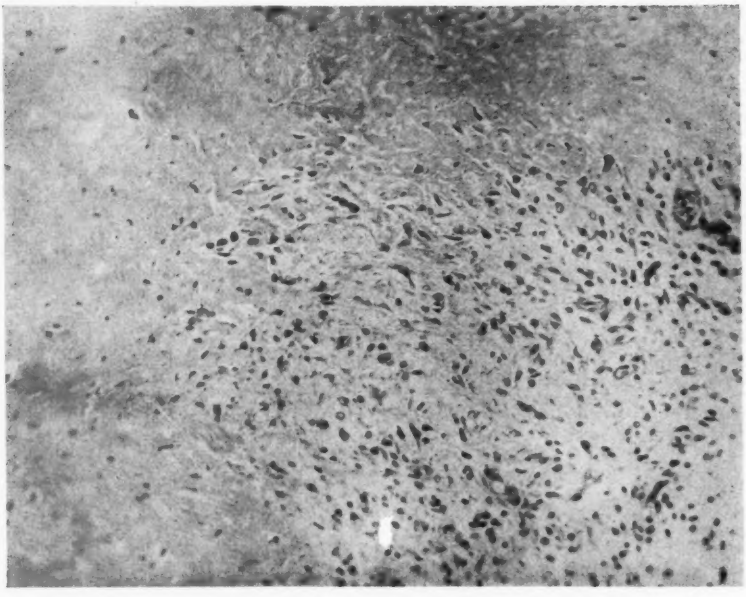
Plate 148

Fig. 1. Case 1. Tissue of organization invading compact fibrin mass in center of vegetation. Hematoxylin and eosin stain. × 175.

Fig. 2. Case 2. Mitral valve with flat vegetation on anterior leaflet and large round mass on auricular surface of posterior leaflet.

A

1



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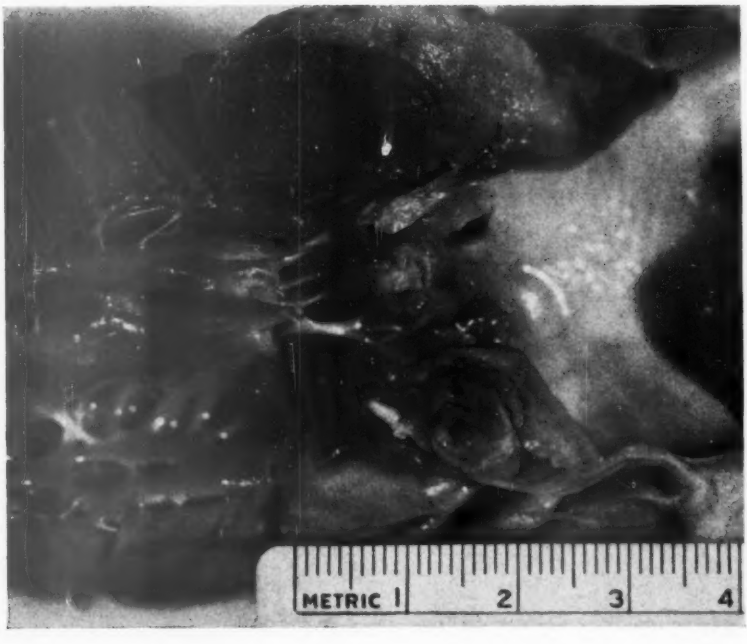

Geiger and Durlacher Endocardial Vegetations Following Penicillin

PLATE 149

FIG. 3. Case 2. Process of organization extending into fibrin mass in center of vegetation on posterior leaflet. Hematoxylin and eosin stain. × 140.

FIG. 4. Case 3. Central portion of vegetation consisting of fibrin, platelets, red blood cells, and leukocytes. Hematoxylin and eosin stain. × 225.

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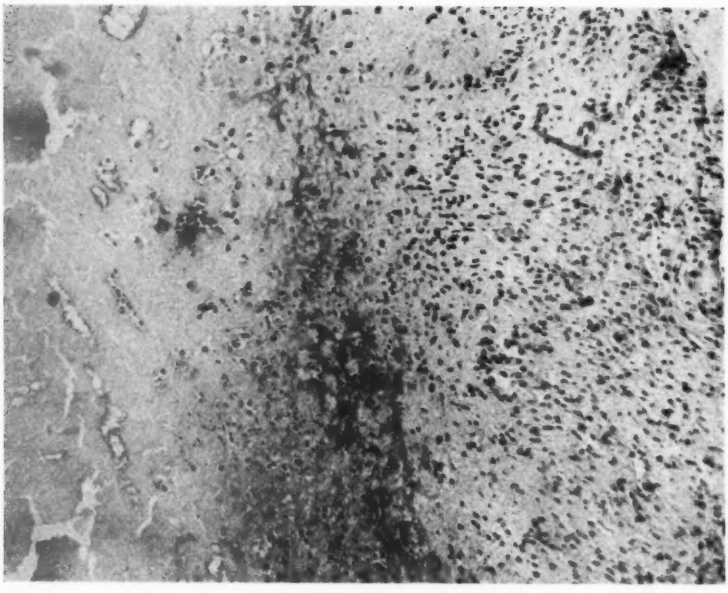
Ge

3



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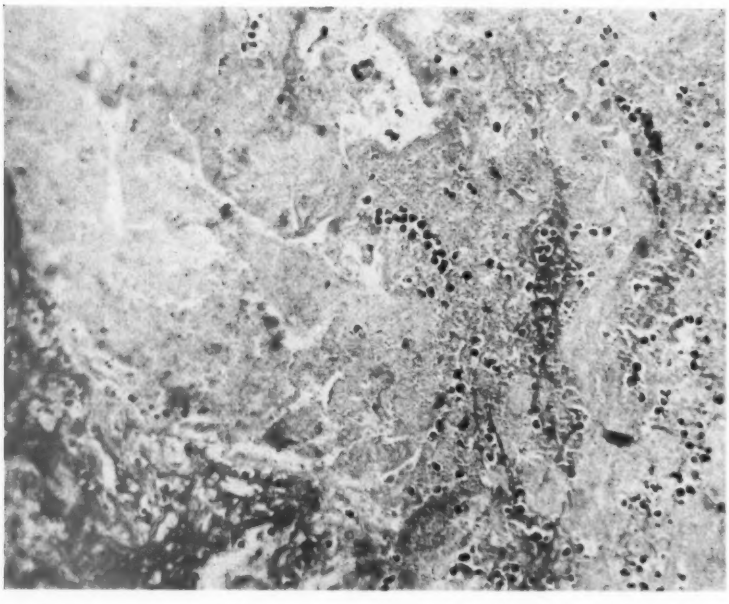


Geiger and Durlacher Endocardial Vegetations Following Penicillin

PLATE 150

FIG. 5. Case 3. Tissue resulting from organization and covered by endothelium on auricular surface of vegetation. There is no evidence of recent thrombosis. Hematoxylin and eosin stain. × 235.

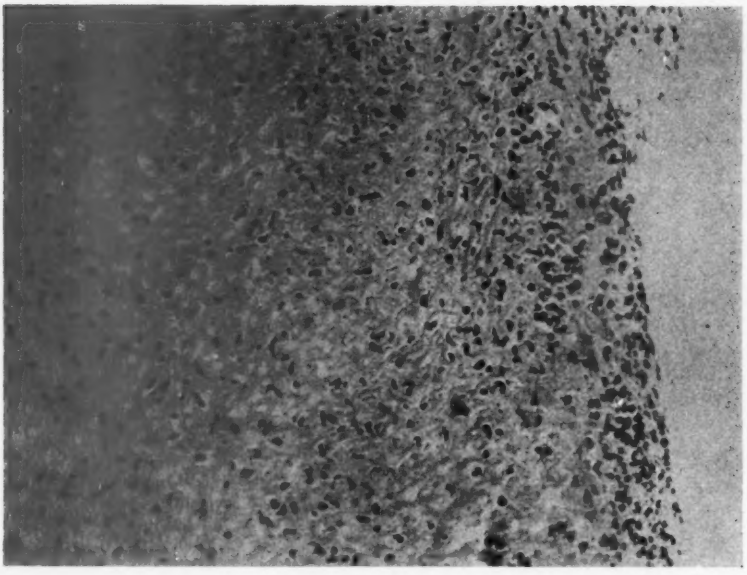
FIG. 6. Case 4. Dense fibrous connective tissue and calcified thrombus on endothelial surface of left auricle above line of attachment of posterior leaflet of mitral valve. Hematoxylin and eosin stain. × 130.

5

6

Geige

5



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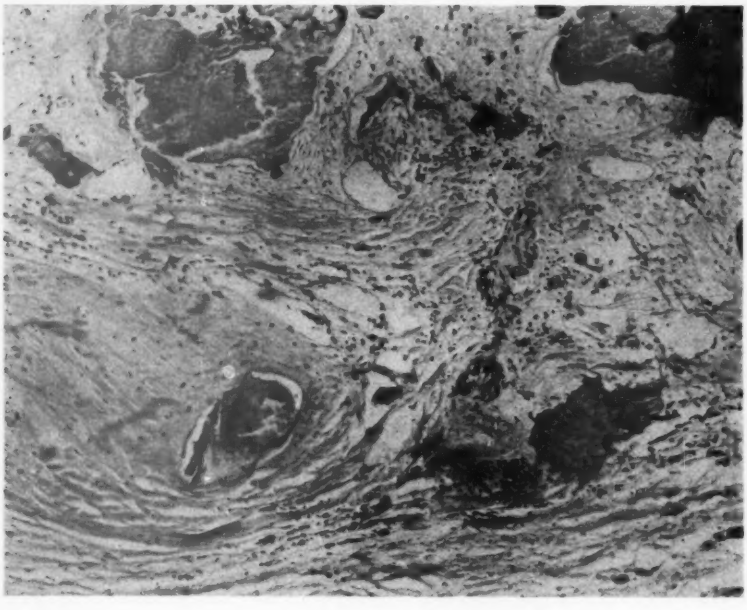

Geiger and Durlacher Endocardial Vegetations Following Penicillin

STUDIES ON EXPERIMENTAL PHOSGENE POISONING

I. The Pathologic Anatomy of Phosgene Poisoning, with Special Reference to the Early and Late Phases *

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Except for sporadic observations, study of the development of the lesions constituting "typical" phosgene poisoning has been neglected, and one of the chief purposes of this report is to close this hiatus. Obviously, a systematic analysis of the sequence of tissue changes from the end of gassing onward is prerequisite to the comprehension of the symptomatology and the abnormal physiology of phosgene poisoning. Another object is to describe both the regressive-reparative and the degenerative processes found in animals surviving the critical 72 hour post-exposure period, which has received more, but still inadequate, attention in the past. The massively edematous lung of acute phosgene poisoning will be treated briefly inasmuch as the present studies add little to what is already in the literature.

It is only in connection with the acute or critical phase of the damage (6 to 72 hours following gassing) that a comparison can be drawn between the findings in men poisoned in industrial accidents or in war and the lesions observed in animals poisoned under controlled conditions. As will be pointed out, there is imperfect agreement between the two, and reasons for this may be traced to one or more of the following: (a) The prevalence of respiratory infections during 1915 to 1920 may have complicated or altered the response of the lung to injury, particularly in men surviving longer than 36 hours. (b) In both accidental and field exposures, it has never been possible to estimate with any reliability the dose to which the victim was exposed, and it is known that exposure to extremely high or low concentrations for a given time will result in anomalous lesions.[1-3] (c) Since phosgene was rarely used in World War I,[4-6] except in combination with some other gas, descriptions of the lesions found in casualties may not be applicable to pure phosgene injury. (d) The conditions existing during the interval between death and autopsy were not specified in the reports on human material; this stands in direct contrast to experimental work. (e) Species differences may, in part, account for variation in response

* The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Pennsylvania.

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to injury. In this connection it may be significant to the interpretation of the cardiovascular abnormalities that the animal is more resistant to anoxia than is man.[7-9] On the whole, however, agreement among the various pathologic data is more satisfactory than that between physiologic experiments and inferences from clinical observation.

The data in this report, it is believed, make it clear that the reaction of the lung to phosgene is a type reaction common to several other injurious agents, rather than a specific response.

Materials and Methods

Exposure of the animals to phosgene was carried out in an 850 liter chamber through which specified phosgene-air mixtures were drawn at rates of 300 to 600 liters per minute, depending on the size and number of animals. The concentration of phosgene in the mixture was sampled by absorbing the phosgene from a measured fraction in an alcoholic solution of alkali, subsequently analyzed for total chloride.

Two technics of exposure were used in these studies; they appear to give very similar, if not identical, clinical and pathologic findings:

(1) The desired concentration of phosgene was built up in the chamber, whereupon the caged animals were inserted into the gas mixture via a double door slide arrangement; after a time predetermined to result in a desired mortality at a given phosgene concentration, the animals were withdrawn into a cleansing air current. The phosgene concentration was sampled synchronously with the exposure. This "push-pull" method simulates field exposures and saves time, but causes the operators to work hurriedly in dangerously high concentrations of phosgene; it is not suited for species such as the rabbit and goat which are able to suspend breathing for a considerable time.

(2) The "rise-fall" method was used mainly because of greater convenience and safety. The caged animals were placed in the chamber and then the admixture of phosgene with inflowing air was begun. This results in an exponential rise of concentration which was calculated and found to reach equilibrium in 10 minutes. After a given time at this equilibrium, the phosgene was turned off, whereupon the phosgene concentration in the chamber fell by the same exponent, but reciprocally. The duration of exposure was taken to be the sum of the duration of constant composition plus 10 minutes. The phosgene concentration was sampled throughout the period of constant concentration.

The phosgene used in these experiments was stated to be better than 99.9 per cent pure $COCl_2$, the remainder being HCl.

The dose of phosgene is expressed conventionally as the product of mean concentration in mg. per liter of air (C) and duration of exposure in minutes (T). This convention is based on the assumption that minute breathing volume, and hence the intake of gas, is proportional to the metabolism which in turn is a function of the weight; it is comparable to the usual dose system based on body weight.[10] Therefore, the adults of a species, regardless of size, are assumed to receive the same proportional dose of a given CT product. To anticipate the results, analyses of the mortality data support this convention; in any event "individual susceptibility" and behavior during exposure are regarded as more important variables.

The dogs, cats, rabbits, and guinea-pigs employed were adults of mongrel origin, whereas the rats, weighing 150 to 225 gm., were of the genetically homogeneous Wistar strain, obtained from the Institute as needed. The latter were secured in an effort to reduce "individual susceptibility" to a minimum. The other animals were not used until an isolation period had shown them to be free of infection and eating well. All of the small animals of an experiment were gassed simultaneously in individual cages, while the dogs were gassed in pairs or in fours in cages.

The dogs were sacrificed by rapid arteriotomy under amytal or local anesthesia and the small animals by thoracotomy under nembutal anesthesia. A number of ungassed animals of each species were similarly sacrificed for comparison.

The animals were autopsied at once after death (with the exception of a few kept overnight at —4° C.) and representative tissues taken for histologic examination. The lungs were removed *in toto* after ligation at the hilum, and multiple sections, or the whole lung in the case of the rat and guinea-pig, were fixed at once in Helley's fluid. The tissues embedded in paraffin were sectioned at 7.5 μ and stained with hematoxylin and eosin or special stains as indicated.

The animals listed in the tables which follow are representative of the larger number which were gassed for histologic study. Further, the data agree with those from more than 1,050 dogs and 2,500 rats used in this laboratory in experiments on phosgene poisoning.

Results

To facilitate description, the course of phosgene poisoning has been divided into three phases, which are somewhat different from those of Winternitz.[11] It must be emphasized that such a division is arbitrary, since the sequence of events initiated by exposure to phosgene is con-

TABLE I

Evaluation of Acute Pathologic Changes in the Lungs of Dogs Exposed to a Moderate Concentration of Phosgene ($L.D._{30}$) by the "Rise-Fall" Technic

Dog no.	Conc. of CG*	Time after "CG on"	Sacrificed (S) or died (D)	Emphysema	Congestion of lung	Alveolar edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight
	(*mg./L.*)	(*hours*)							(× *100*)
118	0.49	0:38	S	++	o, +	o	±, +	±	1.13
117	0.49	1:38	S	++, +++	o, +	±, +	+	+	1.25
529	0.64	1:39	S	+	o, +	+	+	±	1.20
539	0.56	1:52	S	+	o, +	+	+	+, ±	1.60
569	0.52	2:39	S	++, +++	+	++, +++	++, +++	+	2.11
728	0.64	2:46	S	++, +++	o, +	++, +++	++, +++	Dl‡,o	1.36
439	0.82	3:36	S	++, +++	o, +	+	++	o	1.45
551	0.58	3:46	S	++, +++	+, ++	++, +++	+++	±	1.32
642	0.48	3:52	S	++, +++	++	+, ++	+, ++	o	1.82
566	0.58	3:52	S	++, +++	+++	++, +++	++, +++	Dl, o	1.46
579	0.67	4:33	S	+++	++, +++	++, +++	+++	Dl, o	2.24
440	0.45	5:07	S	+++	+, +++	++, +++	+, +++†	Dl, o	1.89
530	0.64	5:31	S	+++	+, +++	+, +++	++, +++	Dl, o	2.80
043	0.37	6:18	D	+++	++	+, +++	+++	o	3.47
443	0.76	7:32	S	+++	++, +++	+++	++, +++	Dl, o	2.69
447	0.76	8:17	D	+++	+++	+++	++, +++†	Dl, o	2.10
550	0.60	8:35	S	+++	++, +++	++, +++	++, +++	Dl, o	3.52

* CG = phosgene. Total theoretical time of exposure = 30 min.
† Beginning "bronchopneumonia" suggested by leukocytic invasion.
‡ Dilated.

TABLE II

Evaluation of Acute Pathologic Changes in the Lungs of Dogs Exposed to a High Concentration of Phosgene ($L.D._{70}$ approx.) by the "Push-Pull" Technic*

Dog no.	Conc. of CG†	Time after "CG on"	Sacrificed (S) or died (D)	Emphysema	Congestion of lung	Alveolar edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight
	(*mg./L.*)	(*hours*)							(× *100*)
815	2.98	0:08	S	+	±	o	o	±	1.04
795	3.30	0:08	S	+	o	o	o	±	1.30
807	3.33	0:20	S	++	±	o	o	±, +	1.19
819	2.95	0:31	S	+	o	o	+	±	0.92
817	2.98	0:33	S	++	o	o	o	±	1.12
818	2.95	1:01	S	+	++	o	+	±, +	0.97
816	2.98	1:04	S	++	+	o	+++	+	1.06
808	3.33	1:42	S	+	+	o	o	+	1.18
796	3.30	2:04	S	++	o	o	++	±	1.51
820	2.95	4:09	S	+++	+	+	++	o	1.82
809	3.33	4:10	S	+++	+	++	+++	Dl§	2.50
849	3.20	4:20	D	+++	+++	+	++	Dl	4.65
797	3.30	5:19	S	++	++	++	+++‡	Dl	2.50
390	3.55	6:14	D	++	+++	++	+++	Dl	5.91
287	3.31	6:20	D	+++	++	+	+++‡	Dl	2.92
243	3.22	7:00	D	+++	+++	+++	+++‡	Dl	4.26
279	3.22	7:05	D	++	+++	+++	+++‡	Dl	4.48
254	3.25	8:54	D	+++	+++	+++	+++	Dl	2.82

* By R. H. only.
† Time of exposure = 3 min.
‡ Beginning "bronchopneumonia" suggested by leukocytic invasion.
§ Dilated.

tinuous and ends either in death or in repair and survival. These three artificial divisions are, chronologically:

1. The incipient phase which extends from gassing up to 2 to 6 hours.

2. The critical phase in which the majority of deaths occur; in survivors this phase ends about 3 days after exposure.

3. The regressive and reparative phase which extends from the fourth day onward; the time of completion of repair is uncertain. The residua of phosgene poisoning are regarded as the terminal stage of repair.

The development of the typical lesions from controlled exposure to pure phosgene has been condensed in Tables I through IV. In the tables o, ±, +, + +, and + + + are to be read as none, doubtful, mild, moderate, and severe, respectively. Each designation represents the independent evaluation of the sections by two pathologists (D. R. C. and R. C. H., Jr.), and where they disagreed, both evaluations were entered. Agreement is remarkably close considering that each entry is the summed evaluation of all sections of the lung of an animal. "CG" is the convention for phosgene.

The method involves a sampling procedure and is susceptible to all of the errors inherent in sampling a population, since a given section may constitute but a small portion of a lung. This is important since it has been observed that the periphery of the lung may not show the same degree of radio-opacity as the hilar region.[12]

The Incipient Phase of Phosgene Poisoning

The pulmonary changes in the incipient phase are those which precede clinically-evident pulmonary edema. They are emphysema, necrosis and sloughing of bronchiolar mucosa, perivascular and peribronchial edema, congestion, thickening of the alveolar membranes, and perhaps bronchial-bronchiolar constriction.

Emphysema. As indicated in the tables, emphysema was the earliest prominent lesion in animals exposed to potentially lethal doses of phosgene. Tables I and II dealing with dogs gassed by different technics indicate that this very early, if not immediate, change is not a technical artifact; the data on the other species confirm this. Not only is it probably the earliest anatomic change, but it appears to attain full development prior to the other changes.

Grossly, emphysema appeared as small pale patches on the surfaces of otherwise unaltered lungs in the earliest specimens. Microscopically (Fig. 1), the distribution of emphysema was patchy and lobular. The alveolar septa were extremely thin and at times fragmented, while the

capillaries were thin and relatively bloodless. Small areas of advanced patchy atelectasis, as seen later, were not apparent in this phase.

Bronchiolar Constriction. The criteria adopted for recognition of constriction of the airways were thickening of the musculature and folding and compression of the mucosa, so as to cause obvious narrowing of the lumen (Fig. 2). On this basis constriction was relatively infrequent (Tables I through IV), but when found it was usually restricted to the finer air passages, in particular, the terminal bronchioles. Of the several species, only the guinea-pig showed conspicuous narrowing. In nearly all instances, the constriction was found only in animals sacrificed within 2 hours after gassing; later the bronchioles were dilated rather than constricted (Fig. 3). Thus, in general, bronchiolar constriction, to whatever degree it did occur, was transient and soon supplanted by dilatation.

Necrosis and Sloughing of the Bronchiolar Mucosa. Swelling of the mucosa of the small and medium-sized bronchioles with sloughing of the epithelium to form plugs in the lumina was found in the earliest stages. It was constantly present, although overshadowed by emphysema. The epithelium sloughed in shreds and irregular patches, and some areas which had not sloughed showed signs of incipient necrosis. The slough together with mucus formed more or less solid masses which were mobile, since a mass might be found to plug the lumen of an intact bronchiole (Fig. 4) or an air passage might be free of all mucous membrane. Such lesions were usually confined to bronchioles proximal to the respiratory bronchioles.

The number of plugged air passages varied from section to section and from animal to animal. Correlation of the location of such plugs with emphysematous lobules was not feasible without serial sections and this was not attempted. The sloughs were believed not to be artifacts.

Perivascular and Peribronchial Edema. Lace-like expansions of the connective tissue around the medium and larger blood vessels and air tubes was another constant early change (Fig. 5). These cuffs were formed by distention of the interstices of the connective tissue with a lightly staining eosinophilic material. These lacunoid spaces were distinct from the lymphatics, which were not particularly distended in this phase. The site of origin of this fluid was not detected, but it clearly was not alveolar because the cuffs faded out as the vessels became finer and were found prior to alveolar edema. Causal relations of this extra-alveolar edema with other anatomic changes could not be established.

Congestion and Alveolar Edema. Dilatation and engorgement of

TABLE III

Evaluation of the Acute Pathologic Changes in the Lungs of Rats after Exposure to L.D.$_{50-100}$ C×T's of Phosgene by the "Push-Pull" Technic

C-T data	Time after "CG on"	Sacrificed (S) or died (D)	Emphysema	Congestion of lung	Alveolar edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight
	(hours)							(× *100*)
	0:16	S	+++	o	o	+, ++	o, ±	1.04
Rat group 38	2:13	S	+++	±, +	±, +	++	o, ±	1.10
C=0.30 mg./L.	4:13	D	+++	++, +++	+++	+++	o, ±	3.46
T=13.5 min.	5:13	D	+++	+++	+++	++, +++	o	3.28
	9:13	D	+++	++, +++	+++	++, +++	o	3.27
	0:15	S	++, +++	o	o	+, ++	o, ±	0.85
	0:25	S	++, +++	++	o	+, ++	o, ±	0.84
	0:50	S	+++	+, ++	+, ++	+++	o	0.91
Rat group 51	1:10	S	+++	++	+	++, +++	o	0.73
C=0.31 mg./L.	1:40	S	+++	+	o	++, +++	o, ±	0.88
T=10.5 min.	2:10	S	+++	++, +++	+++	++, +++	o, ±	2.11
	2:40	S	+++	+++	+++	+++	o	2.11
	5:10	D	+++	+++	+++	+++	o	3.21
	0:06	S	++	o	o	Sw*, +	o, ±	1.10
Rat group 58	0:33	S	++	o	o	Sw, +	o	1.27
C=1.21 mg./L.	1:33	S	+	o	o	±	o	1.21
T=3.5 min.	3:03	S	+++	++, +++	++, +++	++, +++	o	1.60
	4:33	D	+++	+++	+++	+++†	o	3.58
	1:01	S	+++	+	o	+, ++	o, ±	1.01
	1:12	S	+++	+	o	+, ++	o	1.40
	1:27	S	+++	+	o	+, ++	o	1.03
	1:57	S	+++	o	o	+, ++	o	1.18
	2:27	S	+++	+	+	++†	o	1.26
Rat group 59	2:57	S	+++	++, +++	++	++	o	1.46
C=0.082 mg./L.	3:57	S	+++	++	++, +++	++, +++†	o	1.63
T=57 min.	4:57	S	+++	++	+++	++, +++	o	2.59
	5:57	S	+++	++	+++	++†	o	2.12
	5:57	D	+++	++	+++	+, ++	o	3.45
	7:00—12:00	D	+++	+++	++, +++	+, ++	o	4.02

* Sw = Swelling only.
† Beginning "bronchopneumonia" suggested by leukocytic invasion.

TABLE IV

Evaluation of Acute Pathologic Changes in the Lungs of Cats, Rabbits, and Guinea-Pigs after Exposure to an L.D.$_{100}$ of Phosgene

C-T data	Time after "CG on" (*hours*)	Sacrificed (S) or died (D)	Emphysema	Congestion of lung	Alveolar edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight (× *100*)
Cats	0:16	S	+	o	o	o, +	o	0.74
	0:43	S	++, +++	o, +	o	o, ++	o, +	0.99
C = 0.29 mg./L.	1:13	S	+++	o, ++	+	++	+	1.17
T = 13 min.	2:13	S	+++	+, +++	++	+++	o	1.25
"Push-pull"	4:13	S	+++	++, +++	++, +++	++, +++	o	2.92
technic	6:28	S	+++	+++	+++	+++	o	3.35
	8:13	D	++, +++	+++	++, +++	++, +++	o	3.93
Rabbits	*0:40	S	o	+	o	o	o	0.57
	*0:55	S	++	++	o	+	o	0.69
C = 0.62 mg./L.	1:03	S	++	+	+, ++	o, +	o, ±	0.75
T = 35 min.	1:35	S	+++	++	++, +++	+, ++	o	1.46
"Rise-fall"	1:46	D	+++	+++	+++	++	o	1.76
technic	2:06	S	+++	++, +++	+++	+, ++	o	1.56
	2:46	D	+++	++, +++	++, +++	+, ++	o	1.67
Guinea-pigs	0:12	S	o	o	o	o	o, ±	0.78
	0:24	S	+	o, +	o	o	+	0.81
	0:39	S	+	o, +	o	Sw†	+	1.18
C = 0.34 mg./L.	1:09	S	++	o, +	o, +	Sw, +	+	1.13
T = 9 min.	2:09	S	++	+	+, ++	Sw, ++	+	1.54
"Push-pull"	3:09	S	+++	+	++, +++	++‡	o, ±	3.22
technic	3:39	S	+++	+, ++	+++	+, ++‡	o	3.47
	4:24	S	+++	+, ++	++, +++	++‡	o	2.92

* These two rabbits by "push-pull" technic; C × T's were the same.
† Sw = Swelling most prominent.
‡ Beginning "bronchopneumonia" as suggested by leukocytic invasion.

blood vessels became distinguishable first in the capillaries of the alveolar membranes and perhaps the smaller veins. This congestion coincided with, or possibly preceded, the alveolar edema; but definitely followed the changes mentioned above.

Alveolar edema was detected first as a thickening of the septal strands; eosinophilic material in the alveoli was found later. Apparently the fluid distending the septal strands escaped into the alveoli. The tabulated data make it clear that alveolar edema is the last to appear of the several changes of phosgene poisoning.

The lung × 100/ body weight ratios in the tables are customarily employed as measures of the degree of congestion and edema; they indicate here that edema fluid does not accumulate in appreciable amounts until after the other elements of the "typical" phosgene lung are well developed. The upper limit of normal lung × 100/body weight ratios for dogs is approximately 1.25; for other species, 1.0 or less.

Neither bronchiolar plugs nor emphysema could be related spatially with alveolar edema and all lesions in this phase tended to be without preferential localization. There is no way of deciding whether the fluid in a given alveolus arose from surrounding or more distant capillaries.

In animals poisoned by smaller doses of phosgene, identical lesions developed in the order outlined, with equally spotty distribution. The rate of development, however, was appreciably slower and the reaction appeared less intense.

No lesions were found in extrapulmonary tissues in this incipient phase.

Histologic evidence of damage to the capillary endothelial cells was never found in these sections (compare Daly *et al.*[13]).

The Critical Phase of Phosgene Poisoning

The anatomic picture in the critical phase is the well known "phosgene-poisoned lung." Therefore, the points noted below are only those which warrant emphasis.

In animals which died during the first 48 hours after exposure, the trachea was usually filled with froth, while the bronchi contained mainly fluid; this finding is related to the abrupt release of as much as 500 cc. of fluid via the trachea immediately before death. The mucosal surfaces of the trachea and larger bronchi were usually smooth, and pale or slightly pink. Rarely was there free fluid in the pleural cavities.

The lungs were large and coarsely mottled with alternating dark red and pale yellowish patches of doughy consistency and only slightly crepitant. The sectioned lung presented a smooth wet surface from

which fluid escaped freely; the contrasting surface patches extended throughout the lung.

The heart was usually dilated, especially the right side, but other than an occasional subendocardial or subepicardial hemorrhage no gross abnormalities were encountered. The liver, spleen, kidneys, intestines, and bone marrow were dusky with congestion. The adrenal glands occasionally showed small hemorrhages in the medulla or at the corticomedullary junction. At times the brain was somewhat hyperemic and edematous; other lesions were not found. Essentially, therefore, the extrapulmonary organs showed congestion with occasional, irregularly distributed focal hemorrhages.

The microscopic pulmonary lesions of this phase are shown in the last few entries of Tables I through IV. The lesions were identical, regardless of species and the varying conditions of exposure, and appear to represent merely full development of the earlier changes. The pathologic evaluation did not always agree completely with the clinical state, since in addition to two instances in the tables, many animals sacrificed before being *in extremis* showed lesions as fully developed as those in animals which had died.

Most alveoli were filled with an eosinophilic granular material presumed to be from the fluid in the unfixed lung (Fig. 6). This was found in alveoli of the usual size, in emphysematously distended alveoli, and in atelectatic areas. The fluid contained fibrin, sometimes in large amounts, which tended to be condensed along the alveolar walls. The alveolar capillaries were dilated and engorged except in the emphysematous areas where they were relatively empty. Small hemorrhages were found but were never a conspicuous feature.

Necrosis and sloughing of the bronchiolar epithelium were now obvious and the bronchioles were clearly dilated (Fig. 7). The mucous membrane of the larger air passages and trachea was relatively intact, but covered with mucus and scattered polymorphonuclear leukocytes. More intense inflammatory lesions were seen in the bronchioles which contained necrotic epithelium, plugs of necrotic tissue, and exuded cells; such bronchioles sometimes formed the center of an inflammatory focus extending into the adjoining parenchyma (Fig. 8). This early pneumonia (pneumonitis) was variable in extent, sometimes being absent. Generally it was focal, but occasionally it was diffuse with scattered cellular and fibrinous exudate.

In animals which survived until the third day, regression of the edema and congestion appeared to have begun, while the cellular exudate frequently increased in severity and extent. In addition to the types of cells previously found, mononuclear phagocytes began to

be prominent in the alveoli. The pneumonia at this time usually had assumed a more or less lobular pattern and was termed bronchopneumonia. The high lung/body weight ratios were maintained.

Microscopic examination of organs other than the lung confirmed the gross finding of congestion and showed occasional tiny hemorrhages. In every instance the lesions were of the types customarily associated with circulatory derangement.

In Table V are summarized the terminal findings in dogs poisoned by oxygen, vagotomized under local anesthesia, or subjected to drugs or vagotomy before or after gassing. These data show first that the phosgene picture is not specific and secondarily that procedures designed to interrupt or facilitate bronchomotor reflexes fail to influence the pathologic changes.

The Regressive and Reparative Phase of Phosgene Poisoning

Data concerning the regressive and reparative phase were obtained from 121 dogs dying or sacrificed between 4 and 138 days after exposure to doses of phosgene which had killed initially between 70 and 99 per cent of the groups they survived. The majority had served as controls in various experiments but some had been used for innocuous therapeutic trials. The dogs were kept in individual indoor kennels on a diet of Purina "checkers," supplemented by cooked horse meat. Although this diet proved to be adequate for normal dogs, inanition was a frequent finding in deaths during the first month after exposure.

Originally an attempt was made to distinguish a regressive stage from a reparative stage, but this was abandoned when it became apparent that: (a) The two processes overlapped not only from animal to animal, but also within various areas of the lungs of one animal. (b) Dogs which made good clinical recoveries showed earlier predominance of reparative processes and more rapid regression of edema, and vice versa. (c) The pneumonic process which had begun in the critical phase varied in degree and persistence; often it obscured the regressive process and probably interfered with repair. In some instances the pneumonic changes were so severe as to appear to be the immediate cause of death. By the third week after exposure, however, the reparative processes predominated, because animals showing little tendency toward repair had died.

Typically, the regressive changes consisted of a gradual diminution of the amount of fluid in the alveoli and reduction of congestion, so that by the end of 2 weeks after gassing the lungs failed to drip fluid on section. Microscopically, the eosinophilic alveolar material took a deeper stain and by the end of the third week had practically disappeared. The trachea and bronchi appeared normal; the mucosa lining

TABLE V

Evaluation of Pathologic Changes in the Lungs of Dogs Subjected to Various Procedures

Dog no.	C.T. and technic	Procedure	Sacrificed (S) or died (D)	Time after "CG on"	Emphysema	Congestion of lung	Alveol. edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight
	(*mg. per min. per L.*)			(*hours*)						(× *100*)
61	14.6	Bilat. vagotomy* before CG	D	2:35	+, +++	+++	+++	++	o, ±	3.98
2	14.6	Bilat. vagotomy* before CG	D	4:35	+++	+++	+++	++	o	3.95
42	11.0	Bilat. vagotomy* before CG	D	7:13	+++	+++	+, +++	+, ++	o	3.47
164	17.9	Bilat. vagotomy* after CG	D	4:40	+++	+++	+, +++	+, ++	o	2.96
161	17.9	Bilat. vagotomy* after CG	D	5:50	+++	+++	+, +++	+, ++	o	4.23
44	None	Bilat. vagotomy only	D	22‡	+++	+++	+, +++	+, ++	o	3.10
45	None	Bilat. vagotomy only	D	23‡	+++	+++	+, +++	+, ++	o	3.05
50	None	Bilat. vagotomy only	D	12 to 18‡	+++	+++	+++	+, ++	o	3.41
51	None	Bilat. vagotomy only	D	30 to 36‡	+++	+++	+++	+, ++	o	3.04
442	18.6	Atropine† before CG	D	4:39	++	+++	+, +++	+	o	3.14
643	14.5	Atropine† after CG	S	4:00	++	+++	+++	+	o	3.10
798	19.4	Atropine† after CG	D	7:08	+++	+++	+++	+	o	5.02
000	13.7	Eserine† before and after CG	D	6:46	+++	+++	+++	+	o	2.75
909	11.7	Ergotomine† before CG	S	4:14	+++	+++	+, +++	++	o	2.34
913	11.7	Ergotomine† after CG	S	4:01	+++	+++	+++	++	o	1.56
620	None	95+% O_2 for 48:30 hrs.	D		++	+++§	+++	+, ++	o	4.09
623	None	95+% O_2 for 47:00 hrs.	D		+, ++	+++§	+++	+, ++	o	3.95
622	None	95+% O_2 for 48:30 hrs.	D		++	+++§	++, +++	+	o	3.56
621	None	95+% O_2 for 48:30 hrs.	S		o, ±	++	o, +	++	Dilated	1.53

* Performed under procaine anesthesia.
† Drug in dose sufficient to exhibit action.
‡ Time of survival after operation: not gassed.
§ Multiple hemorrhages in alveoli.
|| Beginning "bronchopneumonia" suggested by leukocytic invasion.

the trachea and bronchi either had sustained no damage, or had regenerated perfectly.

Leukocytic infiltration, or bronchopneumonia, generally became more dense during the first week and changed into foci of consolidation. This reached its peak about 10 to 14 days after exposure in sacrificed animals which probably would have recovered; in those which were obviously moribund the infiltration, in general, was heavier and confluent.

In the gross specimen, elevated, irregular, pale gray patches studded the pleural and cut surfaces of the lung, being more numerous on the former. Beads of purulent material could be expressed from these foci; larger patches had the turgor of early abscesses. Microscopically, the lesions, composed of polymorphonuclear leukocytes, lymphocytes, erythrocytes, and fibrin, resembled a purulent bronchopneumonia (Fig. 9). Generally these lesions were situated in connection with a fine bronchiole, the process spreading distally or laterally to involve varying areas of alveolar tissue. Destruction of alveolar partitions was apparent at times, while in other instances the alveolar walls were infiltrated with monocytes and fibroblasts which extended into the alveolar exudate.

The lung/body weight ratios continued high during this pneumonic process. A return toward normal ratios was associated with resolution of the pneumonia or with minimal infiltration. In other areas of the lung, fibroblastic proliferation was active, forming organized masses which frequently obstructed the bronchioles; sometimes this granulation tissue was found to extend along the airways into alveoli (Fig. 10). During this period the abdominal viscera remained somewhat congested and scattered petechial hemorrhages were still to be found.

It was not possible to establish a relationship between the foci of pneumonia and the early mucosal lesions, although both were essentially bronchiolar. The stained sections of the foci failed to disclose bacteria in the exudate or leukocytes; bacteriologic studies were not carried out.

The fibroblastic proliferation noted above appears to be the means of repair of the alveolar structure since in animals which survived for 1 month after gassing, emphysema, atelectasis, and fibrous scars dominated the picture (Fig. 11). The pneumonic process was still to be found, but it became less and less extensive and active with the passage of time. While the pneumonic foci appeared to heal by scar formation, fibroblastic proliferation also took place in areas free of pneumonia. The bronchiolar epithelium commonly regenerated as cuboid or elongated cells (Fig. 12).

Grossly, the lungs of an animal about 60 days after exposure showed

only emphysema. They offered resistance to sectioning which may be ascribed to condensed fibrous tissue. This scarring, together with scattered emphysema and foci of atelectasis, was seen constantly in the sections. Occasional findings were bronchiectasis, especially in the smaller bronchioles, chronic bronchitis, and tiny pulmonary abscesses.

Various combinations of these lesions persisted in dogs sacrificed more than 6 months after gassing and hence may be regarded as permanent residua of phosgene poisoning. Except for moderate emphysema, scars, and occasional small patches of atelectasis, the lungs were grossly normal. The pleural and bronchial surfaces were smooth. In the sections emphysema and fibrous scarring of the bronchioles were present constantly; small, dense nodules of scar tissue were seen less frequently. Epithelial cells, arranged in single, cuboid, or truly cylindrical layers, had regenerated over or through such scar tissue. Some bronchioles were entirely obliterated by scar tissue, and many alveolar walls were thickened by fibrous connective tissue. Occasionally, small areas of atelectasis containing a few lymphocytes were encountered. The most prominent constant change was distention and fragmentation of alveoli into irregularly enlarged chambers; this may be termed chronic lobular emphysema. It is important, however, that even in lungs with the most extensive residua, there were large amounts of apparently normal alveolar tissue.

Summary of the Anatomic Changes Following Exposure to Phosgene

Emphysema, lobular in distribution, was found constantly in animals sacrificed at once after exposure to doses which killed between 70 to 99 per cent of the animals. An equally constant, but less obvious concurrent finding was sloughing of the bronchiolar mucosa with formation of intraluminal plugs. Shortly thereafter there developed perivascular and peribronchial edema which preceded the alveolar edema. Bronchiolar constriction was found regularly in only one of the several species examined and even in this species constriction was soon supplanted by dilatation.

These changes gradually but steadily progressed, producing finally the typical lung of phosgene poisoning. The alveolar edema contained a high content of protein which by analysis was approximately equal to plasma protein levels.[12,14] Leukocytic infiltration, particularly in the bronchiolar regions, began during the early part of this critical phase and became more prominent the longer the animal survived. Frequently, during regression of the congestion and edema, the infiltration gave rise to a picture almost identical with bronchopneumonia.

In animals with the least pneumonitis, repair of the damage was

most rapid and widespread, but even where infiltration was so extensive as to be considered the immediate cause of death, some evidence of repair was found. Repair consisted of fibroblastic ingrowth which finally gave rise to scar tissue; the process apparently proceeded regardless of complicating pneumonitis. The bronchiolar surfaces were relined by cuboid or cylindrical epithelium.

The lungs of clinically recovered animals showed residua consisting chiefly of foci of scar tissue and lobular emphysema, but tiny patches of atelectasis and cellular infiltration occasionally were still present.

Changes in tissues other than the lungs resembled closely those seen in cardiovascular disease, and had no unique characteristics. Petechial hemorrhages were found frequently but were inconstant in distribution and extent. Evidence of primary damage to the heart and central nervous system was lacking.

Discussion

Since there are no data on the lesions in man during the incipient or developmental phase, it is impossible to compare these changes in man and animals. Tentatively, it may be assumed that man would respond like several other species and show a comparable sequence of lesions. The findings reported above are in essential agreement with three recent experimental studies,[15-17] although differences in emphasis on predominance and rate of development of the various lesions are to be found. These differences seem minor, however, in view of variations in histologic technic (dissecting microscope, vital staining) and variation in gassing methods and concentrations. The older reports on animals sacrificed sporadically in this phase provide inadequate information.[2,11,18]

The findings in man during the critical phase are in general similar to those in experimental animals, but certain inconsistencies warrant closer comparison. In the Hamburg disaster,[19] in which pure phosgene escaped, the pulmonary findings were almost identical with those described in our animals, although the cellular inflammatory lesions appeared earlier and the fibrin later than is usual in experimental poisoning. Outside the lung, however, there were found lesions which seldom or never have been recorded in animals: Focal necrosis of the adrenals, thrombosis of large vessels, subarachnoid hemorrhages, cellular degeneration of the gray matter and a generalized hyperemia, said to extend only to the white matter. Other observers have reported "ring-hemorrhages" in the brain.[6,20,21] In contrast, the autopsy reports on two men who died 19 and 22 hours after accidental exposure [22] stressed edema, emphysema, cardiac dilatation, and necrosis with

sloughing of the laryngeal, tracheal, and bronchial mucosa. This extrapulmonary sloughing suggests exposure to extraordinarily high concentrations of phosgene, but death was not sufficiently prompt to bear this out.

The autopsy reports of 105 soldiers of World War I,[6] who were reputedly gassed with phosgene, contain records of pleural effusion; this also has been recorded by others.[23,24] Effusion was noted rarely in dogs and cats, but it was not uncommon in rodents when autopsy was delayed. Other frequent findings were subendocardial and subpericardial hemorrhages, thrombosis of the pulmonary vessels with thrombi attached to the cardiac valves, and edema and extreme congestion of the brain. Hemorrhages in various parts of the central nervous system were seen only in men who died 36 hours or more after exposure. "Bronchopneumonia" was stated to have been present as early as 12 hours after exposure.[6]

These examples are representative of the pathologic details in the literature on phosgene poisoning in man in the acute stage.[1,4,5,18-21,23-28] In general, the pulmonary lesions do not differ essentially from those recorded on animals poisoned under controlled conditions;[2,11,17,18,29,30] the variations in detail and emphasis are probably attributable in large part to the factors outlined in the introduction. The extrapulmonary lesions, with the exception of congestion, seem much more prominent in man than in animals, but it is questionable whether this constitutes a real difference in the reaction to phosgene. Because the extrapulmonary lesions resemble those found in death from circulatory failure, and since circulatory insufficiency from blood volume loss is present in phosgene poisoning, it is illogical to postulate the formation *in vivo* of specific humoral toxic agents which might cause these lesions.[6,17,20]

Records of thrombosis or other obstructions to the pulmonary circulation in both man and animals in the older literature[6,11,31,32] have no counterpart in the data reported here and the reason for this discrepancy is not known. In any event, it is probable that pulmonary obstruction has little if any significance in the pathogenesis of this edema, since, in addition to the negative evidence, we have found that completely heparinized animals follow the typical phosgene course, with the same sequence of changes.[12]

Autopsy data on man in the regressive and reparative phase are fragmentary and described mainly in terms of "bronchopneumonia."[6,11,33] In a man who died 11½ days after exposure[19] the combination of fibroblastic proliferation, macrophages in the alveoli, chronic bronchitis and bronchiectasis, and residual edema is almost

identical with that in dogs which died at a comparable interval after gassing. Also the findings in a man who died 3 months after exposure [34] are very similar to those in dogs dying about 21 days after exposure. Thus it is reasonable to assume that the reparative processes in human survivors take the same course that has been described for dogs. Evidence corroborating this view is the pulmonary fibrosis, chronic bronchial inflammation, and emphysema detected by x-ray and physical examination.[23,35-38]

It will be noted that the concentration of phosgene may be varied reciprocally with duration of exposure by a factor of 10, without detectably affecting the pathologic and physiologic findings. The data bear out the assumptions on which the dosage system is based, which in turn is the basis for a systematic experimental study of phosgene poisoning. The range of reciprocity is probably much greater than 10.

According to the above data, certain physiologic and clinical concepts of phosgene poisoning need revision. The "latent" period of phosgene poisoning, the more or less asymptomatic interval between exposure and the onset of detectable edema, is clearly a fallacy since the anatomic pulmonary damage, begun during gassing, steadily progresses. The clinical problem in phosgene poisoning (and similar toxic agents) must lie in the application of measures designed to minimize its progress. Clearly the time for treatment is before, not after, edema has appeared, but because such therapy is unknown, one is confined to providing whatever symptomatic relief is indicated.

We never have predicted successfully the probable outcome from clinical observations alone. Dogs poisoned by doses of phosgene killing 70 to 99 per cent of the group immediately showed bradycardia, a general lassitude, motor activity of the colon and bladder, a flared chest, and a rapid shallow type of restricted breathing; there was no relation between the severity of these signs and survival. Later when râles could be heard, the tachycardia, leaden cyanosis, asthmatoid breathing, and prostration were merely confirmatory evidence of the degree of involvement of the lungs. However, because the edema fluid is practically plasma, it was possible to estimate the rate of development and ultimate degree of edema by frequent hematocrit determinations. In general it was observed that the slower the onset and rate of hemoconcentration, or the longer the interval between gassing and clinically detectable edema, the better was the prognosis.

The question of bronchoconstriction, or bronchiolar constriction, in phosgene poisoning needs re-examination. In the past, assumption of this condition was based on subjective substernal constriction and similarity of the breathing to that in asthma.[5] Histologic evidence support-

ing this [1,11] was not qualified with regard to the fact that the fixed tissue might not represent the living state. The present data, thus qualified, suggest that constriction, if present, is transitory and not significant in the critical phase of poisoning when the breathing is most analogous to that of asthma. It may be important that in the one species, the guinea-pig, in which histologic bronchoconstriction was seen, the bronchial smooth muscle is arranged like that in man.[89] Experiments on dogs and rats [12] have led to the conclusion that the asthmatoid breathing is not of constrictor origin, since: (a) In hot weather panting may alternate with periods of asthmatoid breathing. (b) Administration of CO_2 immediately converts the breathing into free, rapid, deep movements. (c) Pressure on the larynx removes the expiratory grunt without shortening the expiratory effort. (d) None of the sympathomimetic, parasympatholytic, or myotropic antispasmodic drugs which are effective in asthma exerted more than a transitory effect on breathing. Unequivocal evidence of relaxation of bronchospasm was not seen. Eserine did not exaggerate the asthmatoid breathing, and vagotomy before or after gassing resulted in breathing characteristic of the ungassed animal, slow and deep with prolonged inspiration. Drugs which act on the heart peripherally (and vagotomy) readily modified the reflex bradycardia without materially changing the breathing. The data in Table V show that the characteristic findings were unchanged by the two procedures most likely to accent or minimize reflex bronchoconstriction. The evidence obtained from gassed lung preparations designed to demonstrate bronchoconstriction is equivocal.[2,13,15]

Nevertheless, evidence of subnormal ventilation is found in the transitory arterial anoxemia and the CO_2 retention and respiratory acidosis seen during the incipient phase.[12,16] Two possible causes of this, other than bronchoconstriction, are (a) obstruction of the airways by the plugs of sloughed epithelium and (b) edema of the remaining bronchiolar epithelium, which is supplied by the bronchial artery, in connection with which the earliest edema was noted. The intrabronchial emboli have been observed before [11,18] but were not assigned a significant rôle. Their occurrence, synchronous with the emphysema, suggests a causal relation which has not been demonstrated. In the gassed dog-lung preparation desquamation of the bronchial mucosa has been observed, although bronchial emboli were not described.[13] Experiments with balloon models indicated that the alveoli supplied by unblocked bronchioles are those which would stretch and rupture under negative pressure.[12] It is tempting to postulate that sloughing of the bronchiolar mucous membrane with plugging of the lumen

causes the unbarricaded alveoli to rupture as they follow the expansion of the chest, which is induced during gassing and may be maintained reflexly. When sufficient edema is present another form of bronchial obstruction probably develops which nullifies all inhalational therapy. As the edema fluid moves about via the bronchial system it mixes with air to form a moderately stiff foam which produces a water lock proximal to otherwise functional alveoli.

In the healing process some bronchial plugs are recanalized, but if they should be replaced by scar tissue the result would be atelectasis, as was observed. The emphysema seems to persist much as it first appeared and it is doubtful that such areas are functionally active. This "vesicular" emphysema has been described as a common aftermath of poisoning by any of the pulmonary irritant gases and it has been implicated in the etiology of the "effort syndrome" found in gas casualties.[23,37,40-42] Psychic factors have been assigned the major rôle in this condition [43] but abnormalities of pulmonary ventilation recently have been detected.[44]

It is improbable that the plugs and emphysema bear a causal relation to the edema, chiefly because in exposure to very low phosgene concentrations edema appears without emphysema or significant bronchiolar changes.[3,45] The hemodynamics of the pulmonary circulation are not grossly disturbed in phosgene poisoning [46,47] and so it must be assumed that the capillaries are damaged functionally by the gas. It is obvious, therefore, that this study has merely reaffirmed that available histologic methods are inadequate to demonstrate the abnormal functional state of the capillary endothelium. Capillary congestion does precede the alveolar edema, but there is no reason to assume that congestion causes loss of normal permeability. Because the hemoconcentration was arrested and reversed in dogs 15 to 35 hours after exposure, it is probable that restoration of normal capillary permeability began in that period.

The majority of dogs poisoned with the doses used in these experiments died of respiratory failure before or near the peak of hemoconcentration. The type of death indicates functional disintegration of the central nervous system, prior to that of other essential organs such as the heart. Since movement of plasma into the alveoli is responsible for both the major defects, the anoxic anoxia and the shock-like hemodynamics, it seems beside the point to insist that one or the other of these two secondary features is the more lethal. Moreover, both these defects set up vicious cycles which are interlocking and supplementary. It is of interest, however, that the rabbit, a "wet" animal, dies of

typical pulmonary edema without significant hemoconcentration, and therefore without stagnant anoxia.[12,14]

The resorption of edema fluid appears to take place slowly, presumably because of its high protein content.[48-50] Some fluid is removed by the lymphatics [14,39,51] but the chief mode of regression of the edema seems to be degradation of the protein into diffusible molecules by the macrophages and leukocytes, which were observed to migrate in. It is still not clear whether this inflammatory reaction is of bacterial origin or is a further manifestation of the original chemical damage by phosgene. The different susceptibility of the lungs of dog and man to bacterial invasion warrants caution in extending these interpretations of the inflammatory reaction to man.

In addition to failure of pulmonary function due to residual edema and infiltration, death in the early reparative phase may result from functional failure of key organs such as the kidney, which have been devitalized by the previous prolonged anoxia.[52,53] A few dogs in which death was delayed for 3 or 4 weeks after exposure showed inanition clinically in conjunction with an apparently more gradual progression of the inflammatory response.

This healing process seems to consist of regeneration of a more or less normal epithelial layer and scar tissue formation, regardless of whether the initial damage had progressed into a focal or confluent inflammatory reaction. This method of healing and the residua are common to lungs poisoned by phosgene, chlorpicrin, lewisite, and mustard vapor.[17,54,56] Evidence of a type reaction on the part of the lung is seen also in the fact that the acute lesions of phosgene poisoning are identical in many respects with the acute lung damage produced by exposure to chlorine and chlorpicrin,[11] nitric oxides,[55,56] ketene,[15] high oxygen tensions, and following deep x-ray therapy,[57] bilateral vagotomy,[58] anaphylaxis in certain species,[59] and the hot gasses of the Cocoanut Grove fire.[60]

Summary

1. The pathologic anatomy of dogs and other species was investigated after experimental exposure to median doses of phosgene. Particular attention was placed on the sequence of development of the lesions and on the anatomic changes found in animals that survived the acute or critical phase in which the majority died.

2. Extensive emphysema, sloughing of the bronchiolar mucosa, and questionable bronchiolar constriction were found at once after gassing. Peribronchial edema, pulmonary congestion, and alveolar edema de-

veloped subsequently and in that order. The rapidity of development and extensiveness of these lesions were roughly proportional to the severity of the exposure.

3. Recovery from the massively edematous lung of "typical" phosgene poisoning was found to be primarily a process of resorption of edema and scarring. A moderate cellular inflammatory reaction accompanied this process, but sometimes it became so excessive as to be indistinguishable from bronchopneumonia.

4. The residua of phosgene poisoning consisted of pulmonary scarring, lobular emphysema, and small, irregular areas of atelectasis and bronchitis.

5. Comparison of the data with the scanty and somewhat unsatisfactory data on man leads to the conclusion that the processes in man and animals appear to be similar.

6. The findings reported here indicate that certain present concepts of phosgene poisoning need revision.

7. Study of the pathologic anatomy of phosgene leads to the view that the response of the lung to phosgene is a type reaction common to several other agents.

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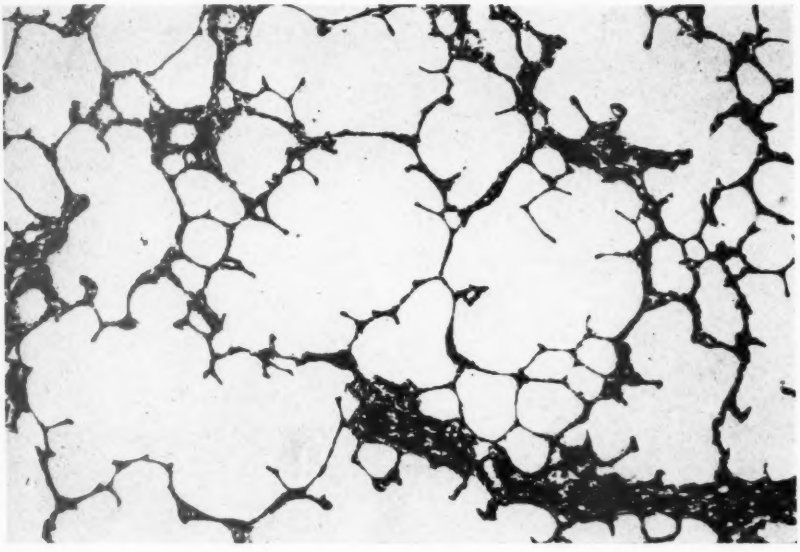
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 151

FIG. 1. Emphysema in the lung of a dog, showing thinning of the alveolar septa. Animal sacrificed 38 minutes after beginning of exposure to phosgene; Concentration of phosgene (C) = 0.49 mg. per liter of air. Duration of exposure (T) = 30 minutes. × 92.

FIG. 2. Bronchiolar constriction. The mucosa is compressed and the lumen is obviously narrowed. Dog sacrificed 8 minutes after beginning of exposure. C = 2.98 mg. per liter; T = 3 minutes. × 92.



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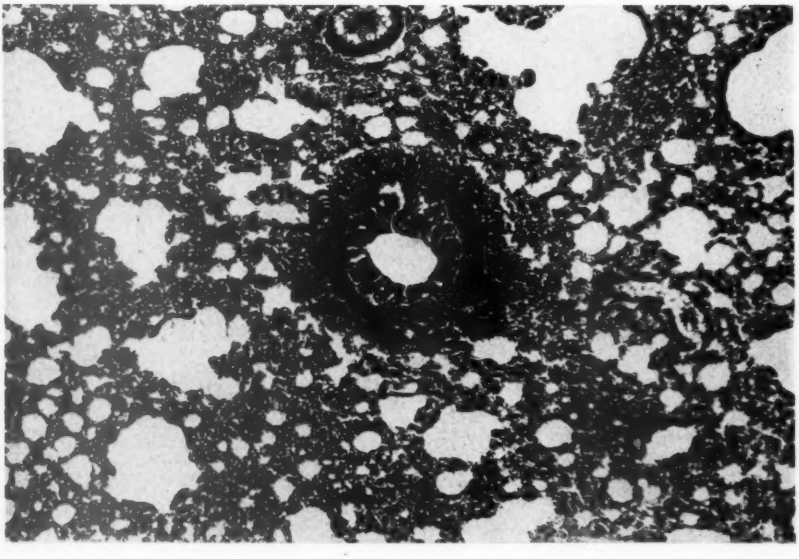

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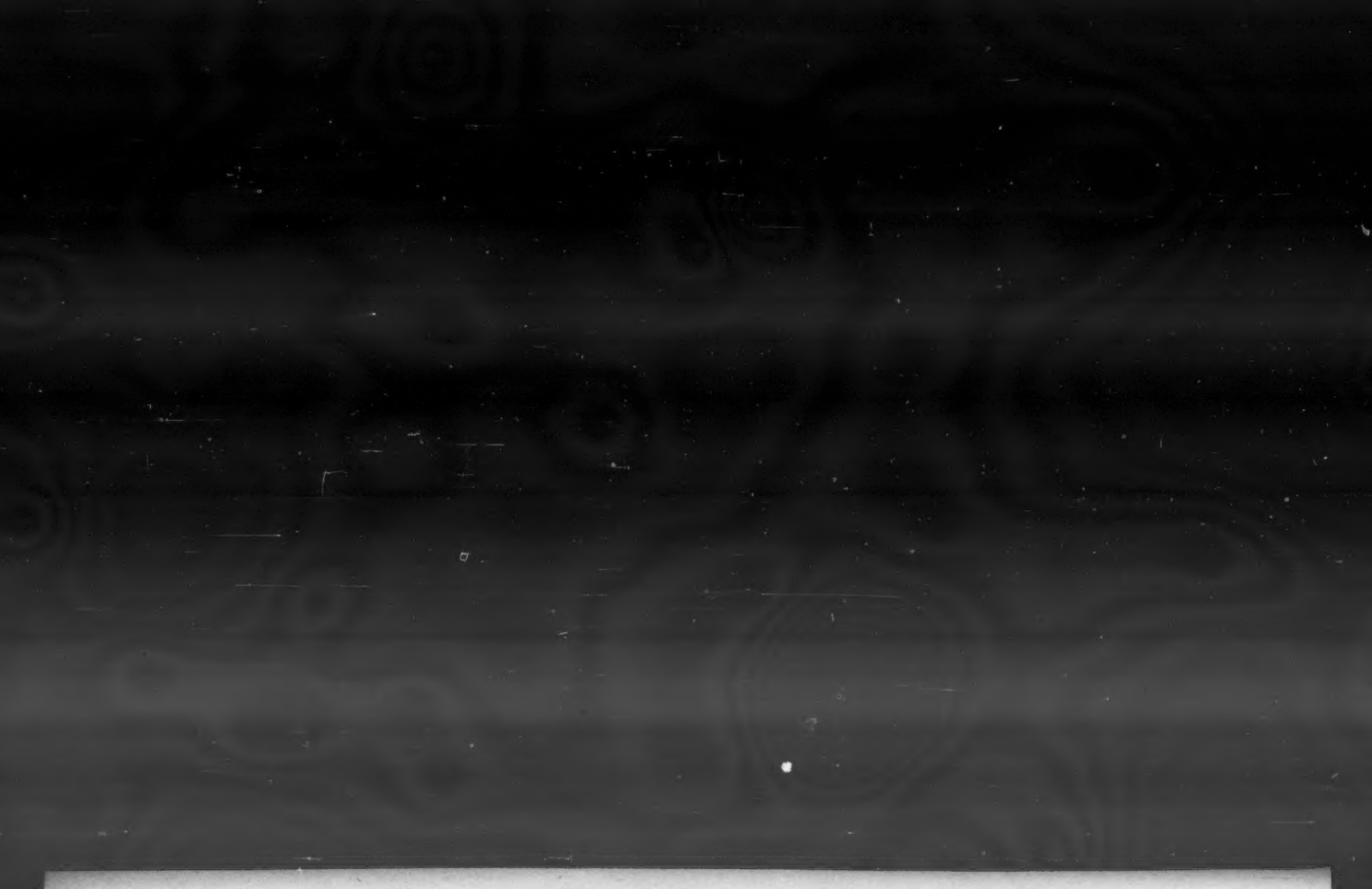
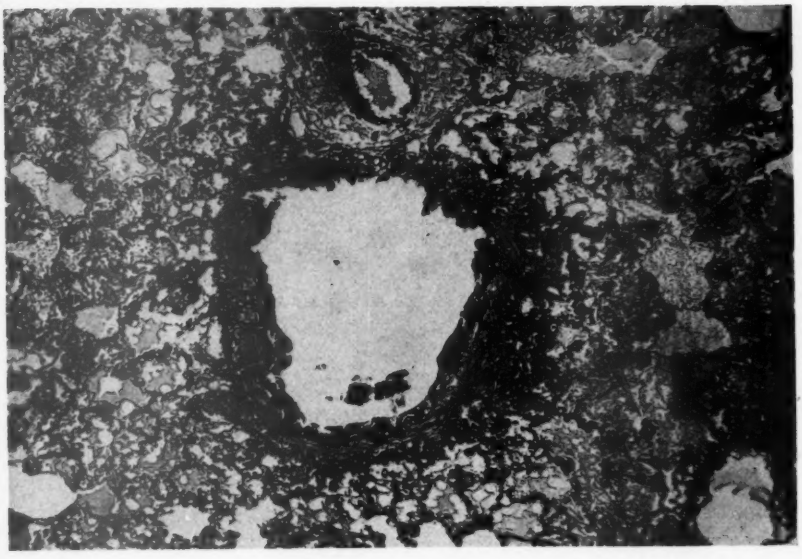
PLATE 152

FIG. 3. Widely dilated bronchiole, found in later stage. The mucosa has sloughed away and the surrounding alveoli are edematous. Dog sacrificed 4 hours and 10 minutes after beginning of exposure. C = 3.33 mg. per liter; T = 3 minutes. × 92.

FIG. 4. Bronchiole containing a plug of desquamated epithelium which has been transported from elsewhere, since the bronchiolar mucosa at this point is still intact. Dog sacrificed 1 hour after beginning of exposure. C = 2.98 mg. per liter; T = 3 minutes. × 200.





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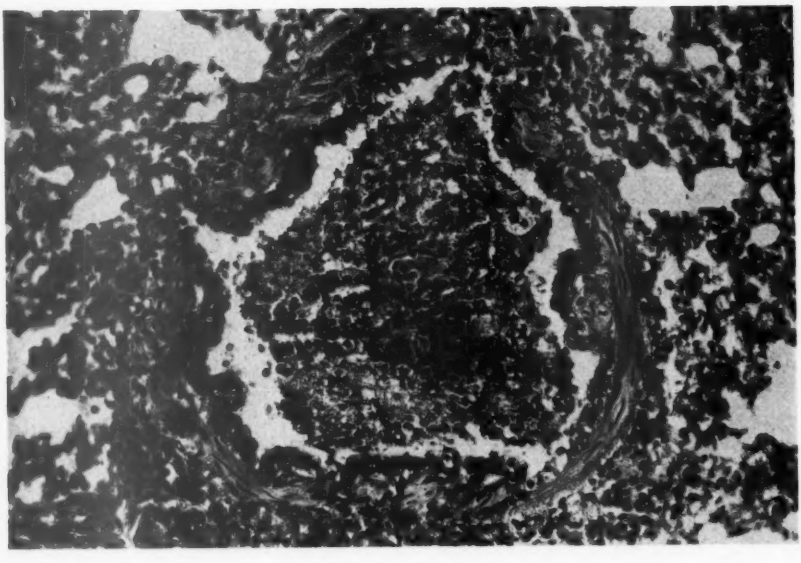

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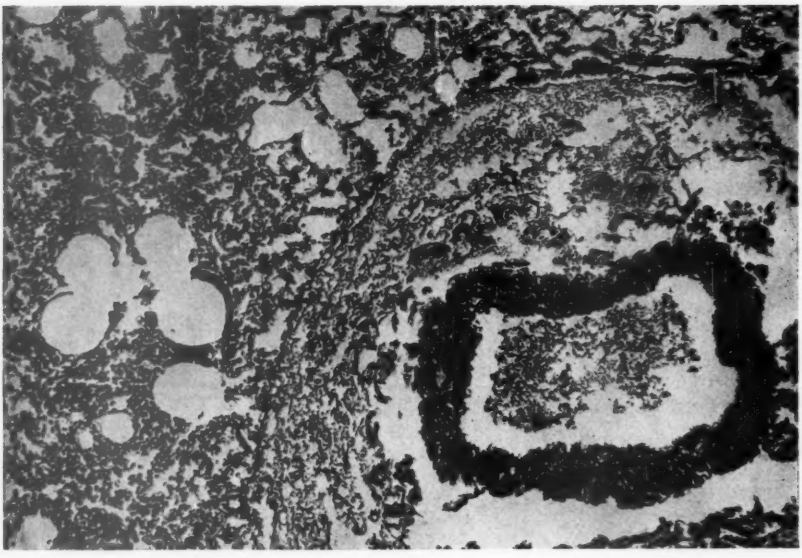
PLATE 153

FIG. 5. A wide lace-like cuff surrounding a vessel, indicative of perivascular edema. The adjacent alveoli contain no fluid. Dog sacrificed 2 hours and 4 minutes after exposure. C = 3.30 mg. per liter; T = 3 minutes. × 20.

FIG. 6. Typical alveolar edema in the critical phase. Of note is the patchy emphysema. Dog died 7 hours after exposure. C = 3.22 mg. per liter; T = 3½ minutes. × 92.



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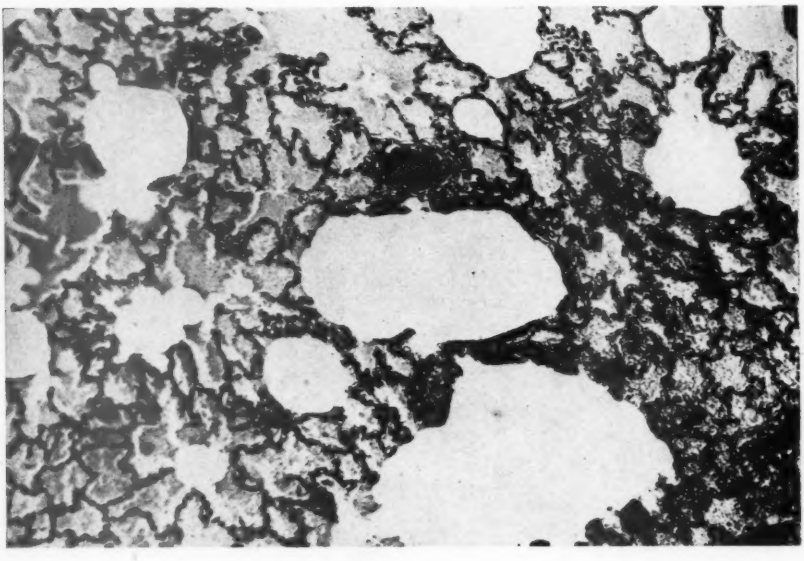

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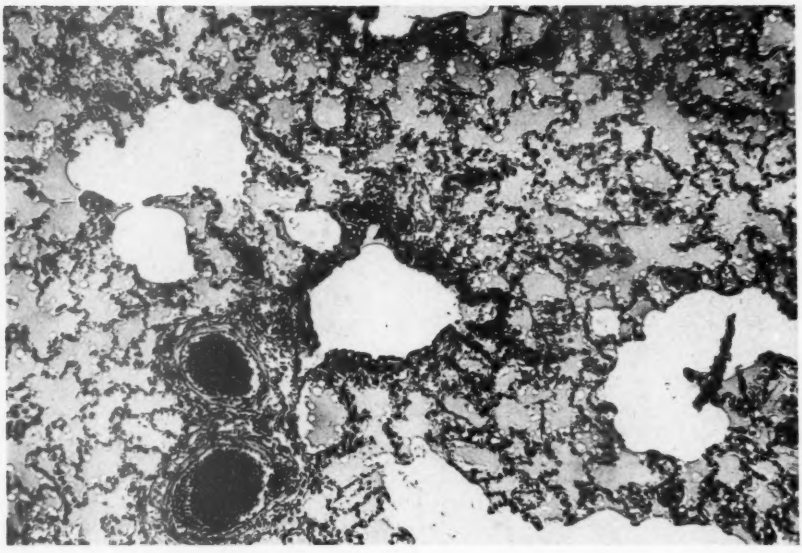
PLATE 154

FIG. 7. A widely dilated bronchus from which the mucosa has sloughed, together with alveolar edema and emphysema, in the critical phase of phosgene poisoning. Dog died 8 hours and 17 minutes after exposure. C = 0.759 mg. per liter; T = 30 minutes. × 92.

FIG. 8. A denuded bronchiole containing polymorphonuclear leukocytes which forms the center of an inflammatory focus extending into the surrounding alveoli. The exudate consists of fibrin and scattered polymorphonuclear leukocytes and lymphocytes. Dog died 12½ hours after exposure. C = 0.263 mg. per liter; T = 30 minutes. × 180.



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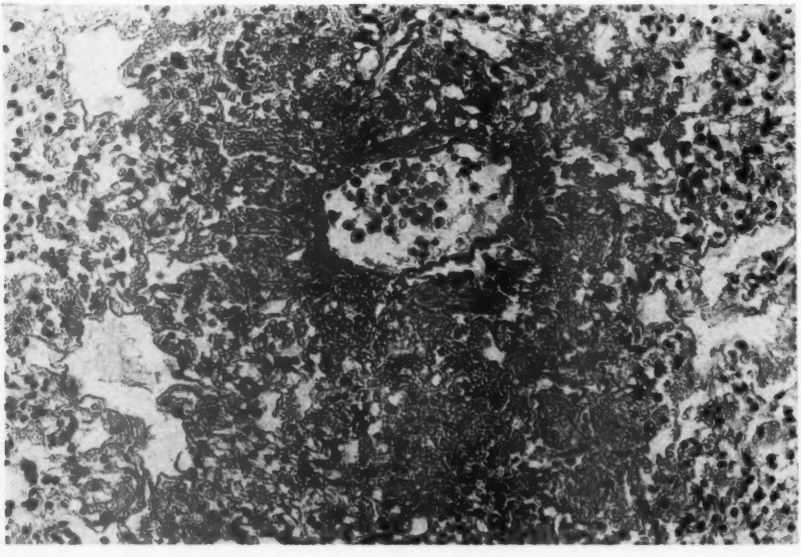

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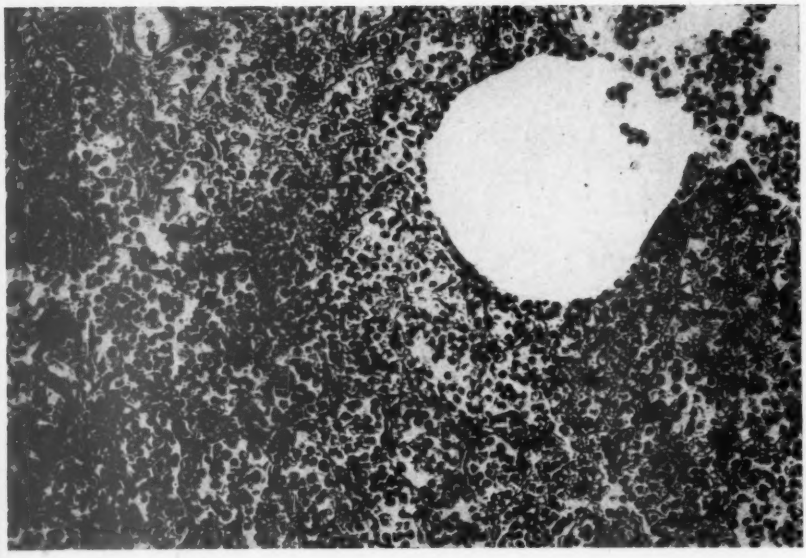
PLATE 155

FIG. 9. Advanced bronchopneumonia in the lung of a dog dying 20 days after exposure to phosgene. The alveoli are filled with polymorphonuclear leukocytes, lymphocytes, fibrin, and a few red cells. C = 0.238 mg. per liter; T = 30 minutes. × 180.

FIG. 10. Granulation tissue replacing alveoli in the lung of a dog that died 72 hours after exposure. C = 0.264 mg. per liter; T = 30 minutes. × 300.



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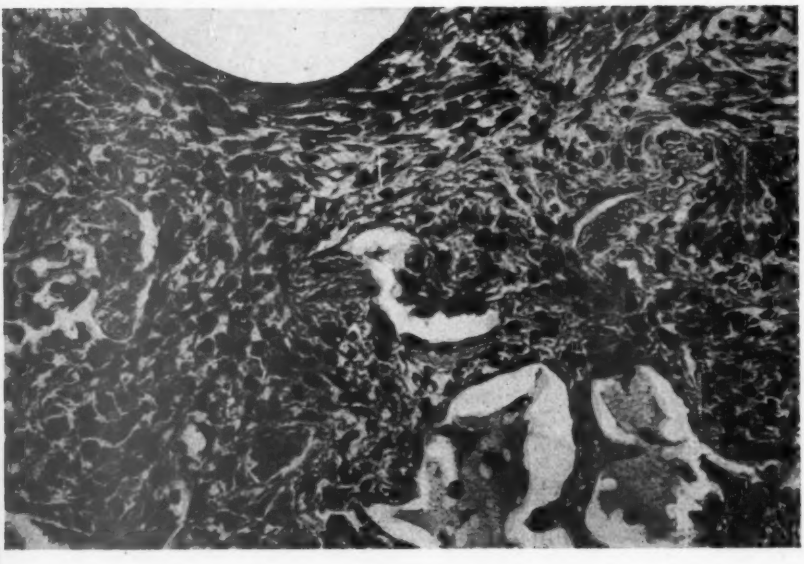

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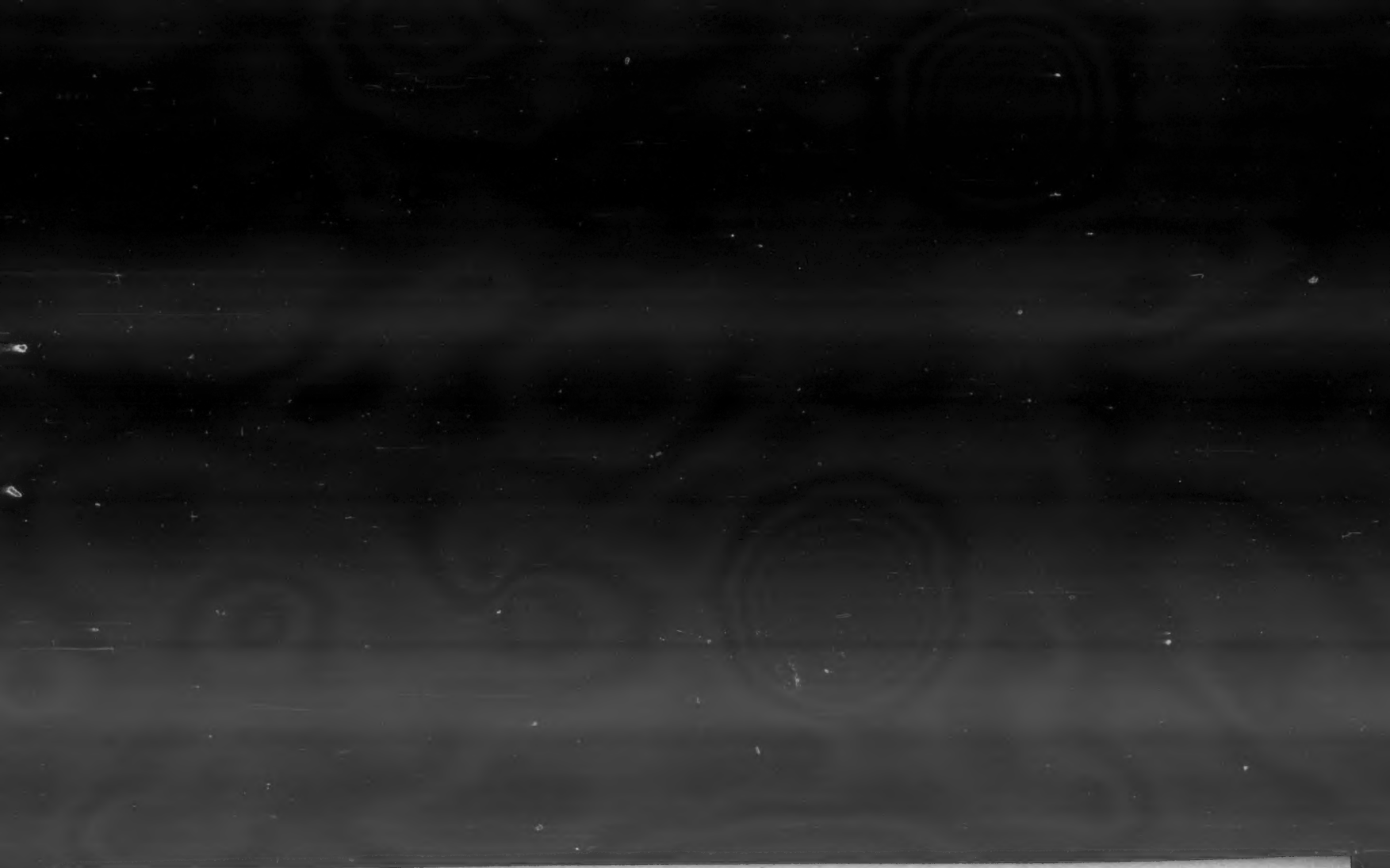
PLATE 156

FIG. 11. Section of the lung of a dog sacrificed 27 days after exposure to phosgene. The emphysema persists and parts of the lung are atelectatic; patches of fibrosis are apparent. C = 0.295 mg. per liter; T = 30 minutes. × 83.

FIG. 12. Regeneration of bronchiolar mucosa. The lining cells are cuboidal in some areas, elongated in others. Dog died 6½ days after exposure to phosgene. C = 0.295 mg. per liter; T = 30 minutes. × 300.



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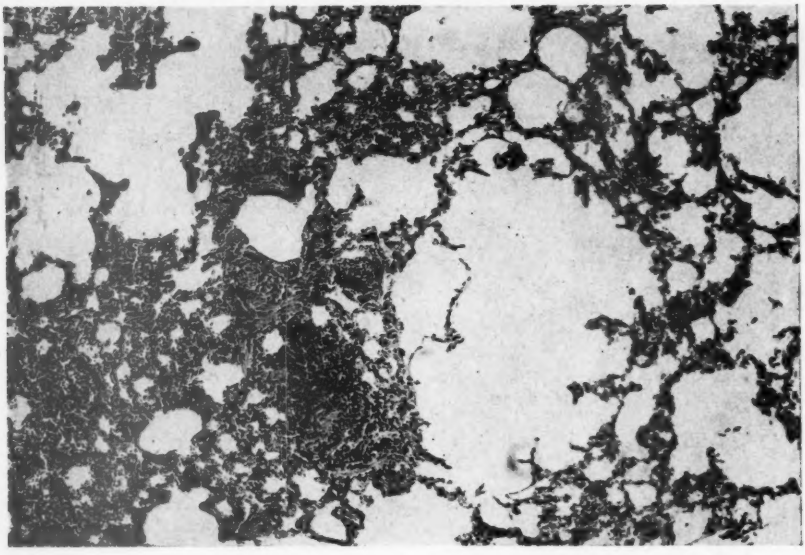

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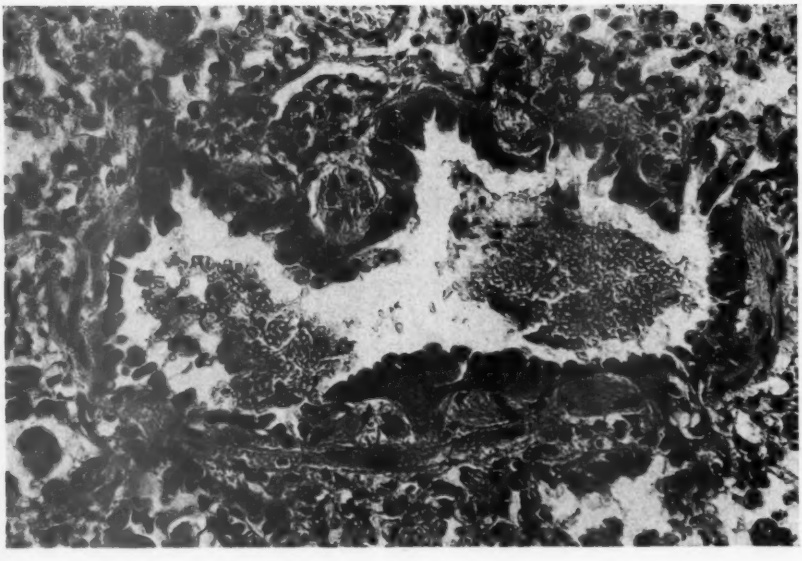

12

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THE PATHOGENESIS OF CONGENITAL POLYCYSTIC LUNG AND ITS CORRELATION WITH POLYCYSTIC DISEASE OF OTHER EPITHELIAL ORGANS

Reconstruction of Cystic Elements in Two Cases *

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(*From the Ayer Clinical Laboratory and the Pediatric Service of the Pennsylvania Hospital, Philadelphia, Pa.*)

Congenital polycystic disease of the lung is hard to define because the lesions are less uniform in structure than in polycystic disease of the kidney, liver, or pancreas and because in adults acquired lesions of the lungs may result in cystic dilatation of the bronchi or alveoli. Thus, there may be uncertainty whether the saccular dilatations of the bronchi seen in bronchiectasis are due primarily to infection acquired after birth or are due to a congenital defect aggravated by chronic secondary infection. There may also be uncertainty in some cases as to whether emphysematous dilatations of the alveoli in adults are acquired or congenital. The difficulty in classification is best illustrated by the list of terms applied to cystic lesions of the lung, compiled by Diamond and Durham,[1] such as pulmonary pneumocyst, bulbous emphysema, alveolar ectasia, cystic bronchiectasis, and open honeycomb lung. Recently a detailed classification of cystic disease of the lung, based on a review of the literature, has been made by Willis and Almeyda.[2,3] They classified the lesions into two large groups. The lesions of the first group are derived from the alveoli and include solitary alveolar cyst, or pneumatocele, and cystic emphysema. Those of the second group derived from bronchi include solitary bronchial cyst and multiple bronchial cysts, or cystic bronchiectasis. Although such a classification is valuable anatomically, it does not differentiate between lesions which are acquired or congenital and thus illustrates further how difficult it may be to decide in a given case whether cystic lesions are the result of a developmental abnormality or of disease beginning after birth. If equivocal cases of emphysema and bronchiectasis in adults are excluded from consideration, however, there is still a group of cystic lesions which are undoubtedly congenital. Thus, there is no dispute that solitary or multiple cysts of the lung manifest at birth or in infancy are the result of faulty development. From a review of the literature it is apparent that in most cases these cysts are derived primarily from bronchi and bronchioles and not from the alveoli.

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When single or multiple cysts found in later life have no communications with the surrounding pulmonary tissue, the origin is usually considered congenital if in and about the lesions carbon pigment is not present. The lack of carbon particles is interpreted as meaning that from birth or infancy inspired air has not entered the cysts. The following discussion will be limited, therefore, to those cases of polycystic disease of the lung occurring in infancy which are definitely congenital.

In congenital polycystic disease of the lung, the lesions vary in size and extent. At times a large solitary cyst occupies most of one lobe and compresses the surrounding parenchyma. More often multiple cysts of varying size are scattered throughout one or more lobes of the lung. The cysts may be completely walled-off and filled with serous fluid or they may be air-containing as the result of small communications with functional bronchi. In addition, many patent bronchi associated with the lesions are irregularly dilated. Such lesions are often described as congenital bronchiectasis. Since the cysts are usually lined by epithelium, often ciliated, they are believed to be derived from bronchi, although in some cases the atria and alveolar ducts without epithelial lining are also described as cystic. When the latter occurs, it is usually spoken of as congenital emphysema. Some of the cysts apparently originate from the smaller bronchi and larger bronchioles, since the walls are partially invested with cartilage and nonstriated muscle and since mucous glands are present in the mucosa. Others are believed to arise from respiratory bronchioles and are composed entirely of a thin fibrous wall lined by flattened epithelium. In many of the smaller cysts and dilated but patent bronchioles, the columnar epithelium is reduplicated and projects into the lumina as papillary folds. Frequently some areas of the lung adjacent to the cysts are collapsed and the development of the alveoli may be less advanced than in the normal portions of the lung. Except in premature infants, however, many of the alveoli among the cysts are normally mature, lack a visible epithelial lining, and appear aerated.

When the lesions are extensive but the child survives after birth, infection of the dilated bronchi and cysts communicating with bronchi often causes fatal pneumonia and suppuration. This outcome is especially apt to occur when cystic lesions of the pancreas are present also. Recently the importance of these associated lesions has been emphasized in the pediatric literature (Andersen,[4,5] Wolman,[6] and Menten and Middleton[7]). It is reasoned that malnutrition resulting from deficiency in the external secretion of the pancreas makes the child liable to infection of the cystic lesions of the lung. When infection occurs, the manifestations at autopsy of acute or chronic inflammation are superimposed on the antecedent congenital lesions.

The fundamental cause of congenital polycystic disease in general is still unknown. As in other organs, the pulmonary lesions have been studied widely in order to determine the anatomic defect which initiates the changes resulting in single or multiple cysts. Yet there is no agreement on the manner in which the lesions are formed, probably because of uncertainty in many instances as to whether the lesions were actually congenital.

As long ago as 1880, Grawitz [8] thought that fetal bronchiectasis and cysts resulted from faulty anlagen and dilatation of lymph vessels. Stoerk [9] and Bernstein [10] believed that the irregular dilatation of the bronchi was secondary to stenosis caused by inflammation of the fetal lung or to excessive growth of the fetal mesenchyme surrounding the bronchi. Syphilis was suggested as a possible cause by Sandoz.[11] Buchmann,[12] Hueter,[13] and Scheidegger [14] believed that a tumor-like proliferation of the epithelium lining the bronchioles caused stenosis and saccular dilatations of the bronchi. In his extensive review of the literature up to that period, Koontz [15] concluded that stenosis of the bronchi or bronchioles is the essential lesion and that dilatation of the bronchioles and air spaces distal to the obstruction is secondary. King and Harris [16] suggested the possibility that local failure of canalization of the developing bronchioles associated with canalization distal to the lesions could account for isolation of cysts and stenosis with bronchiectasis of other bronchi. In a careful study of the cystic lung of a newborn premature infant, Wolman [17] concluded that buds from the trachea became pinched-off during embryologic development. Independent differentiation of these buds was thought to form a closed system of cysts.

Although careful studies of the polycystic lung have been published, little effort has been made to relate the findings with those in polycystic disease of other organs, such as the kidney, liver, and pancreas. In this connection, by means of serial sections and reconstructions, Norris and Herman [18,19] studied the lesions of polycystic kidney and we have studied those of polycystic liver [20] and pancreas.[21] In each organ the essential lesion appeared to be segmentation of the nephrons or of the biliary or pancreatic ducts, as the case may be, followed by isolation of the segments as cysts. Some of these cysts remain small, but others become widely dilated by cystic enlargement. There are indications that in many instances epithelial proliferation causing focal dilatation of tubules precedes the segmentation, but in others segmentation and isolation occur before cystic enlargement. Segmentation, however, does not occur until the epithelial unit is well differentiated and does not prevent the continued normal differentiation of the unit peripheral or distal to the lesions. This process is believed to be a degenerative

one and to be analagous with the normal resorption of the mesonephros, of the first generations of nephrons in the metanephros, and of many of the early intrahepatic bile ducts.

The present study was undertaken to determine whether the lesions occurring in the polycystic lung resemble those in the kidney, liver, and pancreas and whether the genesis of the lesions appears to be identical in all of the organs. Two infants were chosen for examination since the cystic lesions of the lung were unquestionably congenital and since, as previously emphasized, the pathogenesis of polycystic lesions can be more readily evaluated early in the course of the disease.

Materials and Methods

Case 1 * (autopsy no. 3898) was previously reported in detail by Wolman.[17] Briefly, the child was a stillborn Negro infant weighing 915 gm. and measuring 30 cm. in length. The age was considered to be 6 lunar months. Case 2 (autopsy no. 6489) was a full-term white infant, weighing 2550 gm. and measuring 49 cm. in length, who died 4½ hours after delivery of clinically unexplained respiratory failure. In both cases, the significant lesions found at necropsy were confined to the lungs and the remaining organs will not be described.

At autopsy all tissues were fixed either in Regaud's or Kaiserling's solutions. Blocks were embedded in paraffin and stained with either Delafield's or Harris' hematoxylin and eosin. In addition to ordinary sections cut at 5 μ, in each case several hundred serial sections, 15 μ in thickness, were cut from two cubical blocks about 1.5 cm. on a side. Cystic lesions and distorted bronchi and bronchioles were studied and traced through these sections. Some of the lesions were reconstructed by the method previously described.[18,19]

Case 1

Gross Examination. The right lung occupied most of the thoracic cavity. The heart was displaced to the left, laterally and dorsally, and the left lung was small and compressed. Except for atelectasis the left lung was also microscopically normal. The enlargement of the right lung was caused by diffusely scattered, smooth-lined cysts, varying in size from 1 mm. to 1 cm. in diameter. These have been previously illustrated.[17] Although the cysts were completely isolated from the main bronchi, small circular communications were demonstrated between many of them. The cysts were filled with colorless watery fluid which could not be expressed on pressure. In the dorsal part of the lung was a zone of dense parenchyma, resembling the collapsed left

* Dr. Wolman has kindly consented to our use of his case in the present study.

lung, in which no cysts were seen. The right bronchus and its three main branches were patent but the latter ended abruptly and blindly in the cystic tissue. Smaller bronchi could not be traced.

Microscopic Examination. The large cysts were composed entirely of thin layers of fibrous stroma lined by a single layer of flattened epithelium. In some the epithelial lining was lacking. The walls of the smaller cysts were thin and fibrous and these were lined by a single layer of columnar epithelium, often ciliated. Only in areas near the hilum was smooth muscle visible in the walls. The muscle fibers were thin and irregular and usually did not completely encircle the cysts. Also there were but a few small atypical islands of cartilage which likewise did not encompass the cysts. Most of the smaller cysts were irregularly oval or circular. In serial sections, all of the cysts which were traced were found to be completely isolated from the rest of the bronchial tree, but anastomoses between cysts were common. In addition, many outpocketings and relatively undilated branches projected laterally from the cysts. Instead of rebranching into numerous subdivisions in continuity with the ramifications of the air spaces, these branches ended abruptly. Along their courses, however, were many small atypical channels of communication with the terminal air sacs (Fig. 1). Among the cysts were a few, small, undilated bronchi or bronchioles which also were lined by columnar epithelium and which, when traced, were found to end blindly at the proximal ends. These likewise communicated with the air sacs by irregularly spaced slit-like apertures and distally often ended bluntly without subdivision. The terminal air sacs were nearly circular and were expanded. Some contained freshly extravasated erythrocytes. The air sacs were lined by single layers of cuboidal epithelium which was not reduplicated and which did not form papillary projections. The cytoplasm of the cells was more translucent than that of the epithelium which lined the cysts and undilated bronchi. The stroma about the cysts and air sacs was composed of relatively loose connective tissue in which elastic fibers could not be demonstrated with Verhoeff's stain. The number of capillaries appeared normal and many of them were pushing through the walls of the air sacs and the lining epithelium to lie exposed in the air spaces. Nowhere was there evidence of inflammatory exudate (Fig. 2). Two of the cysts have been reconstructed to show the globular contours, the bluntly-ending outpocketings, and the occasional anastomoses (Fig. 5).

In the spongy part of the lung, previously referred to, there were no cysts. This area was surrounded and encroached upon by the cystic portions of the lung. A central core was made up of compact fibrous

tissue in which large blood vessels divided and fanned out toward the periphery. Encircling this core was a mass of collapsed air sacs which also were lined by cuboidal epithelium. Highly irregular but collapsed bronchioles were scattered throughout the parenchyma (Fig. 3). The lining epithelium was columnar and ciliated. There was no investment with smooth muscle or cartilage. Many bronchioles were traced in serial sections and all terminated blindly in at least one direction, but all of them ended distally in collapsed alveolar ducts or atria. Anastomoses between the proximal ends were numerous (Fig. 4). To illustrate the irregularity and patternless arrangement of the bronchioles, several have been reconstructed (Fig. 6).

Case 2

Gross Examination. The great vessels and heart, which lay entirely in the right thorax, were completely transposed. The right lung, although collapsed, was otherwise normal grossly and microscopically. The upper lobe of the left lung was entirely replaced by a large globular cyst filled with serous fluid. The lining was smooth but the wall was irregularly thickened with fibrous trabeculae. The bronchus of the upper lobe was small and ended blindly in the wall of the cyst. In the lower lobe of the left lung the large bronchi were normal and unobstructed. This was the only lobe of either lung which was expanded and air-containing. No gross lesions were seen.

Microscopic Examination. The wall of the large cyst in the upper lobe of the left lung was composed entirely of irregularly thickened fibrous tissue in which small blood vessels were still visible. A single layer of flattened epithelium lined the cyst. There was no evidence of inflammation.

In the lower lobe of the left lung, which appeared normal grossly, the larger bronchi were not remarkable. The medium-sized bronchi were slightly irregular and the cartilages were not symmetrically spaced. Trabeculae of loose fibrous tissue partially surrounded a few of these bronchi (Fig. 7). Many but not all of the distal bronchi and bronchioles, however, were distinctly abnormal. These bronchi were atypically spaced and small saccular dilatations were numerous. Cartilages were absent, but strands of muscularis usually completely encircled the bronchi beneath the mucosa, and also extended laterally to anastomose with the muscular layers of other bronchi. The epithelial lining was uniformly columnar and ciliated and was often thrown into regular folds as if by contraction of the muscularis. When traced in serial sections, none of the bronchi and bronchioles were obstructed or interrupted. Distally the bronchioles repeatedly subdivided and com-

municated with the complex of air spaces by narrow slit-like apertures. On the other hand, there were numerous short lateral branches which did not subdivide normally, but which also communicated with the air sacs by narrow channels. The alveoli in this lobe were diffusely expanded and many of them contained erythrocytes and necrotic squamous epithelial cells. In most areas an epithelial lining was not visible. When present, the lining was composed of a single layer of flattened epithelial cells which usually was detached from the alveolar wall. Fetal mesenchyme was almost completely lacking among the alveoli and the alveolar capillaries were supported only by elastic tissue and a delicate reticulum. There was no evidence of inflammation (Fig. 8). The saccular dilatations and irregularity in contour of the smaller bronchi are illustrated in the reconstruction (Fig. 9).

Discussion

The two cases differ in certain details. In case 1 diffusely occurring cysts, apparently of bronchial origin, were completely isolated from the main bronchial tree. Scattered among the cysts were undilated bronchioles, the proximal ends of which also did not communicate with the main bronchi. In the dorsal segment of the lung collapsed bronchioles and air sacs which showed no evidence of cystic dilatation also were completely isolated. By contrast, in case 2 a solitary large cyst completely replaced the upper lobe of the left lung. Many of the bronchioles in the lower lobe of this lung, however, were highly irregular in contour as the result of numerous saccular dilatations, but so far as could be determined were not broken up into isolated segments.

In both lungs, however, the terminal air sacs or alveoli were remarkably normal in appearance and were mature for the age of the fetus. Although atypically situated, numerous open communications existed in both cases between the bronchioles and the terminal air sacs. Likewise in both cases, the mesenchyme was normally mature for the age of the fetus and evidence of inflammation was lacking. Except for the upper lobe of the left lung in case 2 in which no alveoli were seen, the air sacs about the cystic lesions were expanded in both cases. The immature alveoli in the cystic portion of the lung in case 1 appeared to be fully expanded, but were collapsed in the noncystic dorsal area. This expansion was thought to be slightly in excess of normal physiologic canalization. For an explanation of this phenomenon, it will be recalled that both the larger and smaller cysts intercommunicated and that the smaller cysts and undilated bronchioles had numerous branches interpreted as atria, which communicated with the alveolar complex. Since the larger cysts were filled with fluid, it is quite

likely that, as the result of communicating channels, the air sacs were likewise distended with fluid. The lack of elastic tissue in the enveloping mesenchyme also would favor expansion of the alveoli under these circumstances. In the dorsal part of the lung, in which there were no cysts and in which no fluid presumably had accumulated, compression by the surrounding cysts resulted in collapse and arrested development of the bronchioles and air sacs. A different situation existed in the lower lobe of the left lung in case 2. In spite of the saccular dilatation of the bronchi in this lobe, there was no apparent obstruction of the bronchial tree and many of the alveoli contained aspirated squamous epithelial cells. The expansion of the alveoli was therefore physiologic.

Although the two cases thus show both similarities and differences, it is thought that the underlying defect is the same. If this supposition is true, the slight irregular dilatation and distortion of the bronchioles among the cysts of the right lung in case 1 and in the lower lobe of the left lung in case 2 may be considered the first stage in the development of polycystic disease. The appearance of the lesions suggests that the focal dilatations were caused by expansion of the single layers of epithelial cells which lined the bronchi. However, it has been previously emphasized that segmentation may at times antedate dilatation of the isolated segments.[18-21] In this connection, furthermore, it should be emphasized also that segmentation of the bronchioles is believed to occur only after differentiation of the epithelium. This assumption is supported by the fact that the epithelium lining the dilated segments and cysts was columnar and often ciliated. If the progress of the disease is arrested in the stage of focal dilatation without segmentation, the lesions may be called fetal bronchiectasis. Such lesions may well be the origin of some cases of bronchiectasis in later life. When the lesions progress beyond the stage of focal dilatation, slightly dilated segments of bronchi or bronchioles become pinched off to form the gradually enlarging cysts found in the right lung of case 1 or the large solitary cyst in the upper lobe of the left lung in case 2. Even though partially or wholly disconnected from the main bronchial tree, the air sacs and alveoli distal to the bronchial lesions continue to differentiate in a relatively normal manner.

This course of events is compatible with the steps in the normal development of the lung according to Miller,[22] Norris, Kochenderfer, and Tyson,[23] and Patten.[24] The lungs originate from a ventral laryngotracheal outgrowth of entoderm which divides into two bronchial buds at about 4 mm. At the beginning of the second month, or at about 8.5

mm., the main bronchi are established. After that, the ramifications of the bronchial tree and the terminal air sacs are formed by successive divisions, either dichotomous or monopodial, until differentiation is almost complete at the time of birth. During this period the terminal air sacs are lined by continuous layers of epithelium until shortly before birth so that differentiation and subdivision of the terminal elements of the bronchial tree are always concerned with epithelial structures. Since it has been emphasized previously that segmentation of an epithelial structure is a degenerative or resorptive process, it is altogether likely that focal dilatation and isolation of segments of bronchi in the present cases occurred only after normal proliferation and subdivision was proceeding distal to the lesions and was not seriously impaired by the lesions.

In the kidney (Kampmeier *et al.*[25-30]) and liver (Lewis [31]) it is known that the early generations of nephrons and intrahepatic bile ducts, respectively, are normally resorbed. In the lung, however, it has not been established that any bronchi and bronchioles are normally provisional. Norris, Kochenderfer, and Tyson,[23] however, in a study of the normal lung found evidence in a 15 cm. fetus which suggested to them that some elements of the developing lung may regress and be resorbed, perhaps as the result of the asymmetry of the relatively fixed thoracic cage. If this observation is correct, precedent for the type of degeneration described in the polycystic lung may be present in the development of the normal lung.

For the polycystic kidney, Teuscher [32] suggested that an over-production of excretory tubules, of which some become isolated as cysts, might explain the origin of polycystic disease. Such a theory would be supported by the frequent association of polydactylism with polycystic lesions of the internal organs. The appearance of an excessive number of epithelial elements contributed to the belief of Brigidi and Severi,[33] Nauwerck and Hufschmid,[34] and Staemmler [35] that polycystic disease is a true fetal neoplasm or cystadenoma. Although Norris and Herman [19] could not demonstrate an excessive number of renal tubules in the kidneys which they studied, we [20] did find that the number of intrahepatic bile ducts in a case of polycystic liver appeared to be greater than normal. However, this phenomenon might well be due to the inherent tendency of bile ducts to regenerate following functional and anatomic disturbances. In the right lung of case 1, the number of bronchial elements also appears to be excessive. This appearance (Fig. 1) may be due to the isolation of bronchial segments which, when expanded, tend to occupy more of the parenchyma than is normal. Since

the alveoli continue to differentiate in the presence of bronchial obstruction it is also possible that isolated segments of bronchi may continue to differentiate and branch after their separation from the main bronchial tree. Such a phenomenon would account for the apparently increased number of bronchial elements and the anastomoses between cysts and bronchi (Figs. 5 and 6). It might also explain why isolated segments of bronchi remain as gradually enlarging cysts instead of disappearing. Furthermore, when sections of the lower lobe of the left lung in case 2, in which there were no isolated bronchial segments, were surveyed, the number of bronchi and bronchioles did not appear to be excessive. Consequently, there is as yet no definite evidence that an excessive formation of epithelial elements precedes the lesions of polycystic disease.

In regard to the large solitary cyst in the left lung of case 2, Hepler [36] showed experimentally that in the rabbit kidney obstruction of the papilla did not result in cystic enlargement of the organ. When the arterial supply and the papilla were both obstructed, however, large solitary cysts of the kidney were apt to occur. In the present case it will be recalled that the heart and great vessels were completely transposed. Although the arterial supply of the right lung was not adequately studied at autopsy, it is quite possible that a circulatory anomaly may have been responsible for the large cyst in the upper lobe. Furthermore, it may be said that in general the etiologic significance of deficiencies or abnormalities of circulation in polycystic disease has not been studied widely.

Since the lesions of polycystic lungs are therefore similar to those which have been described in other epithelial organs, the sequence of anatomic changes proposed for the lungs is identical with that previously suggested for the kidney, liver, and pancreas. In all of these organs, the functional elements are generally distal to their points of attachment to the rest of the body and are connected with the body by ducts or stalks which function only as communicating channels. In general, the lesions of polycystic disease, consisting in segmentation either with or without focal dilatation, originate in these ducts. In the normally provisional mesonephros, likewise, physiologic resorption of the organ begins by segmentation of the collecting ducts before degeneration of the glomeruli is initiated (Felix [37]). Thus by analogy it appears that in the polycystic organ the segmentation of the ducts is also a manifestation of resorption or degeneration. It is concluded, therefore, that in polycystic disease the affected organ is more or less abnormally provisional as the result of an unexplained defect in fetal development.

Summary

The polycystic lesions in the lungs of two infants were studied and reconstructions of the cystic elements were made from serial sections.

The fundamental lesion appears to be focal segmentation preceded or followed by focal dilatation of the small bronchi and bronchioles.

If the disease becomes arrested in the stage of focal dilatation without segmentation, bronchiectasis results which may persist for the duration of life.

When the bronchi are broken up into isolated segments, some of these segments persist as gradually enlarging cysts. The well known lesions of polycystic disease are thus established.

The lesions and sequence of anatomic changes in the polycystic lung are similar to those previously described in the polycystic kidney, liver, and pancreas.

In general, the lesions of polycystic epithelial organs primarily affect the nonfunctioning system of ducts.

By analogy with the process of physiologic resorption in normally provisional organs or parts of organs, it is concluded that polycystic disease is a pathologic manifestation of normal fetal resorption and degeneration. The polycystic organ is therefore partially provisional.

The fundamental defect which initiates the developmental anomalies is unknown and the etiologic importance of anomalies of circulation in polycystic organs has not been determined.

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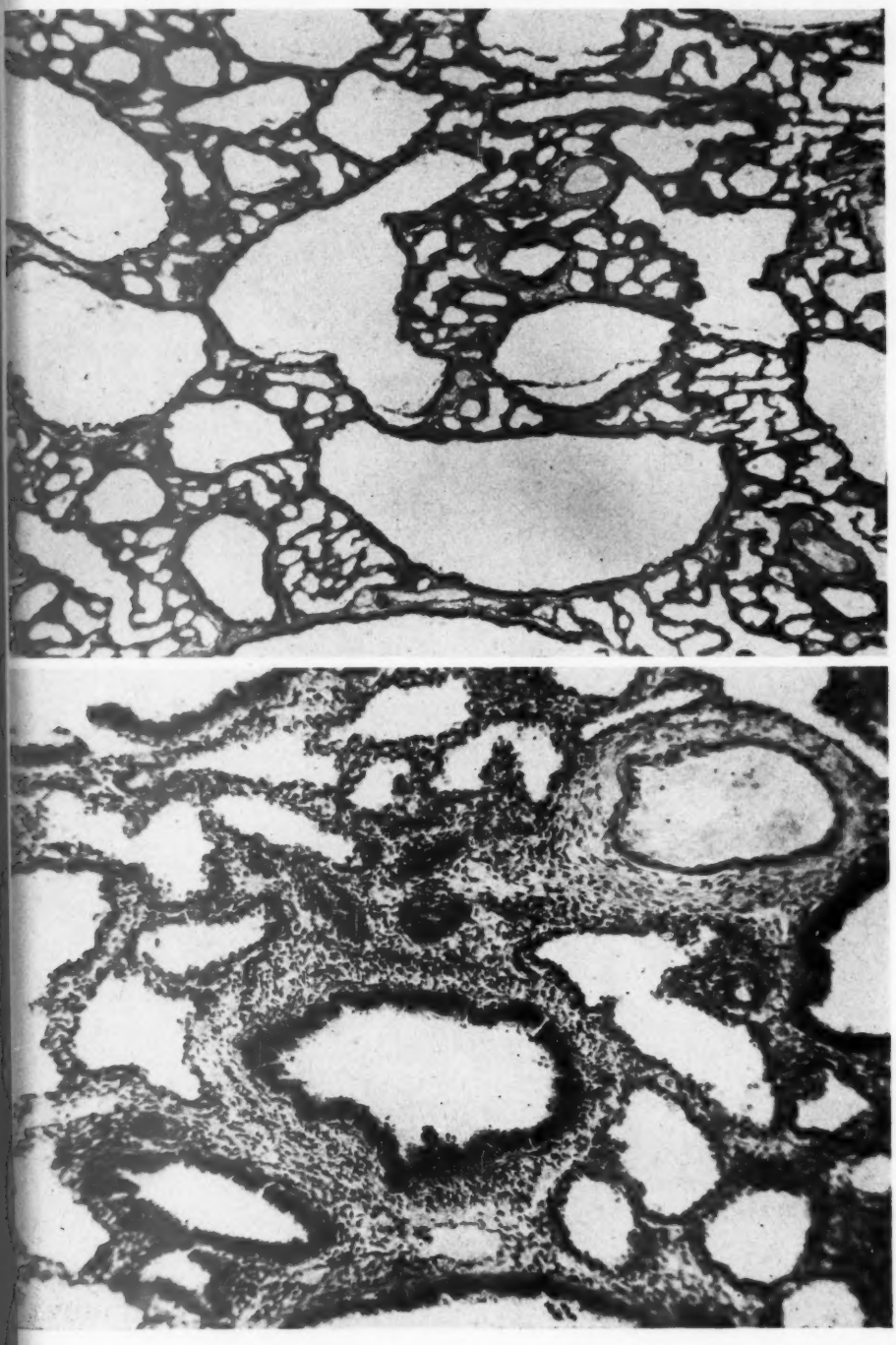
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 157

FIG. 1. Case 1. Cystic portion of right lung. Photomicrograph shows small, irregular cysts lined by single layers of ciliated columnar epithelium. Among the cysts the air sacs are uniformly expanded. To the right of center are three undilated bronchioles and a small artery. × 45.

FIG. 2. Case 1. Cystic portion of right lung. Photomicrograph shows the character of the darkly staining columnar epithelium of the bronchioles in contrast with the translucent cuboidal epithelium which lines the air sacs. In the lower left corner, the slit-like breaks in the bronchial epithelium are atypical communications with the air sacs. × 185.

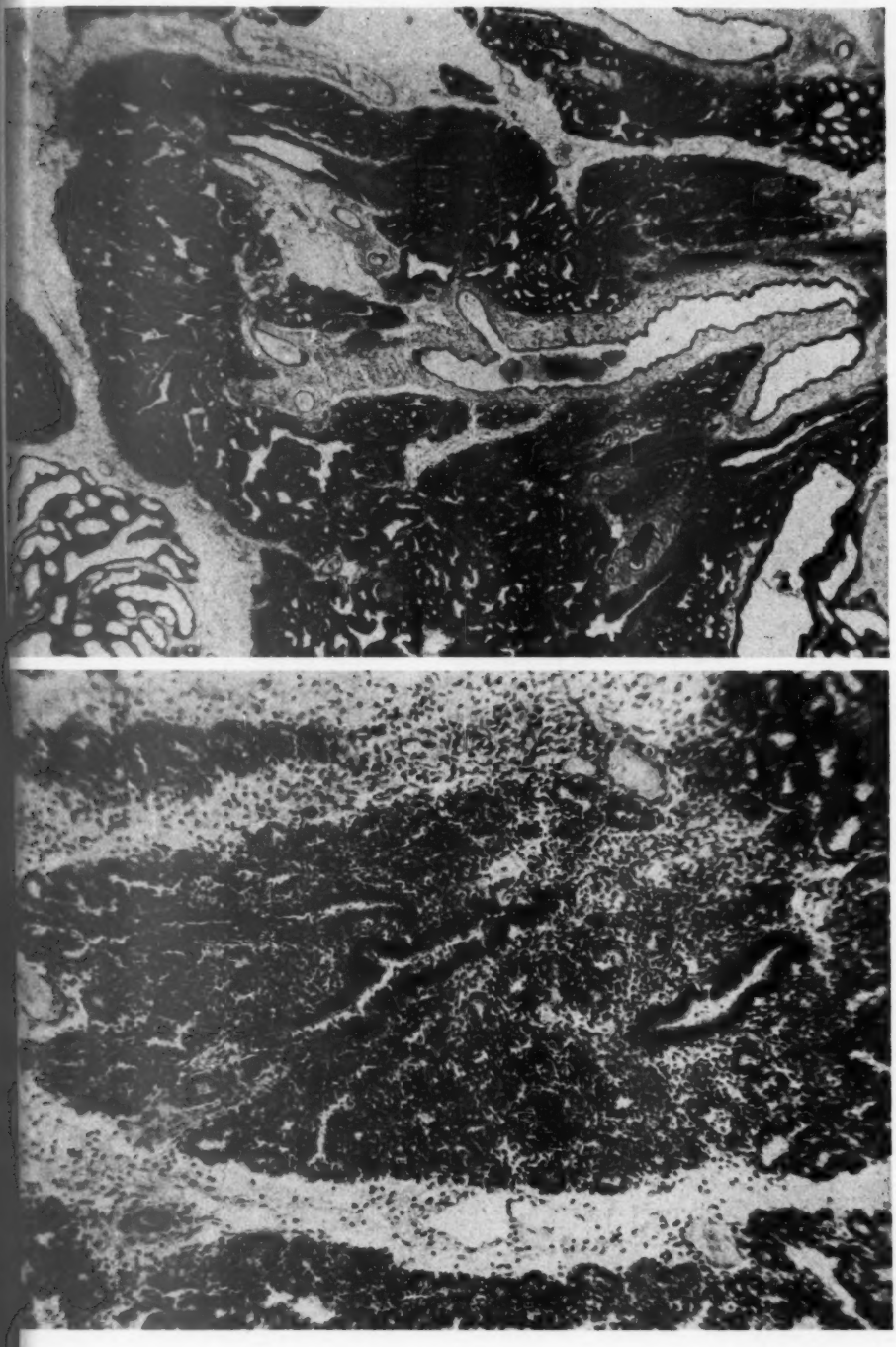


Norris and Tyson Congenital Polycystic Lung

PLATE 158

FIG. 3. Case 1. Atelectatic portion of the right lung. Photomicrograph shows a fibrous central core in which large blood vessels branch toward the periphery. The bronchioles are small and irregular. In the lower left center there is an anastomosis between two bronchioles. In the lower left corner, the cystic portion of the lung is visible. × 45.

FIG. 4. Case 1. Atelectatic portion of right lung. Photomicrograph shows that the air sacs lined by single layers of translucent cuboidal epithelial cells are collapsed. Communications between the two bronchioles in the center and the air sacs are seen at the points where the darkly stained epithelium ends abruptly. × 185.

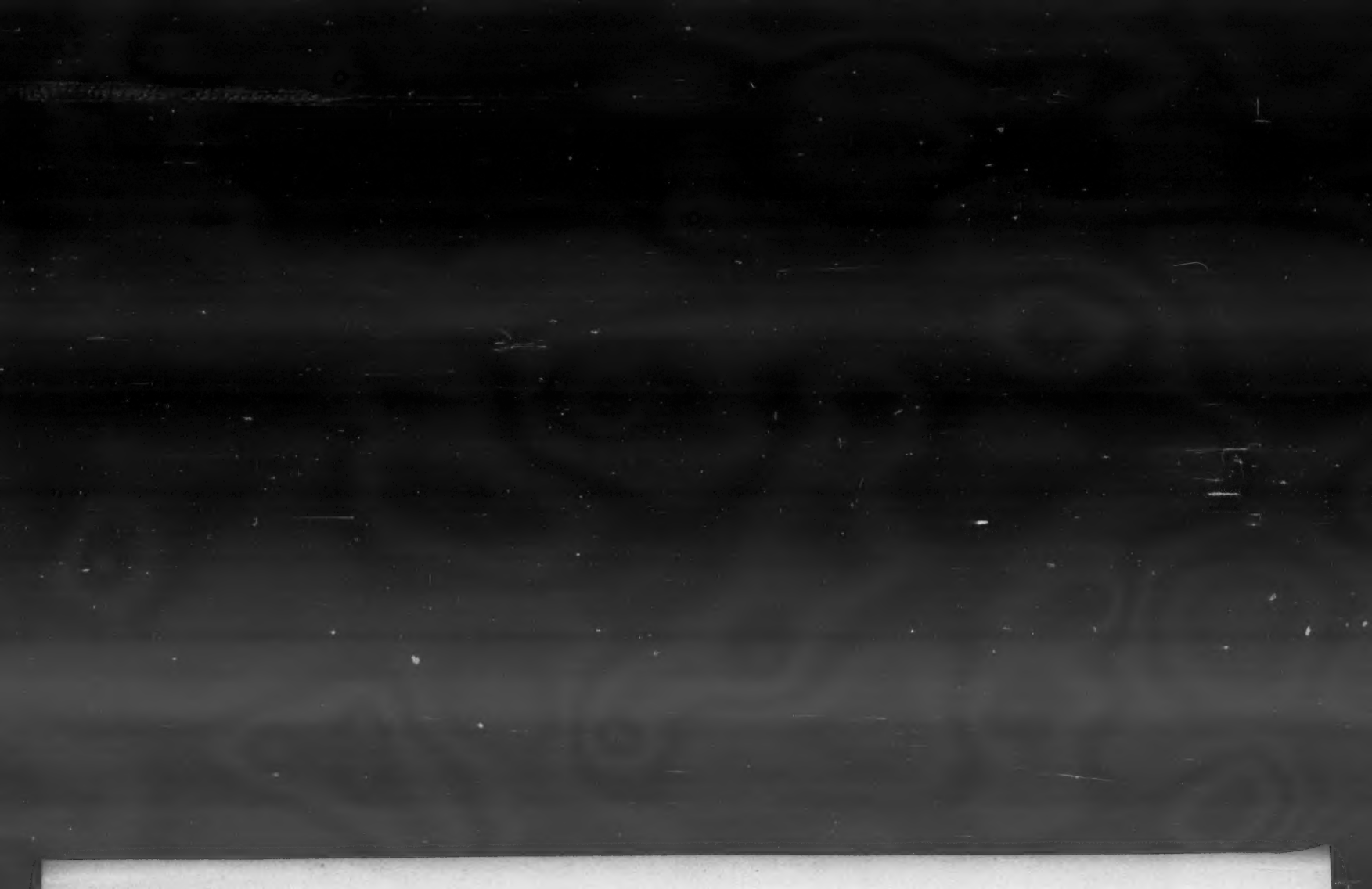


Norris and Tyson Congenital Polycystic Lung

PLATE 159

FIG. 5. Case 1. Cystic portion of right lung. Reconstruction shows the dilatation and anastomosis between two small cysts. Several atypical undilated branches and outpocketings which show varying degrees of distortion are illustrated. Not shown are the communications between these branches and the air sacs. The approximate vertical extent of the reconstruction in the lung was 4.53 mm.

FIG. 6. Case 1. Atelectatic portion of right lung. Reconstruction shows the irregularity of the undilated bronchioles, the atypical distribution, and the anastomoses between them. The shaded background represents collapsed air sacs. Not shown are the communications between bronchioles and air sacs. The approximate vertical extent of the reconstruction in the lung was 0.98 mm.



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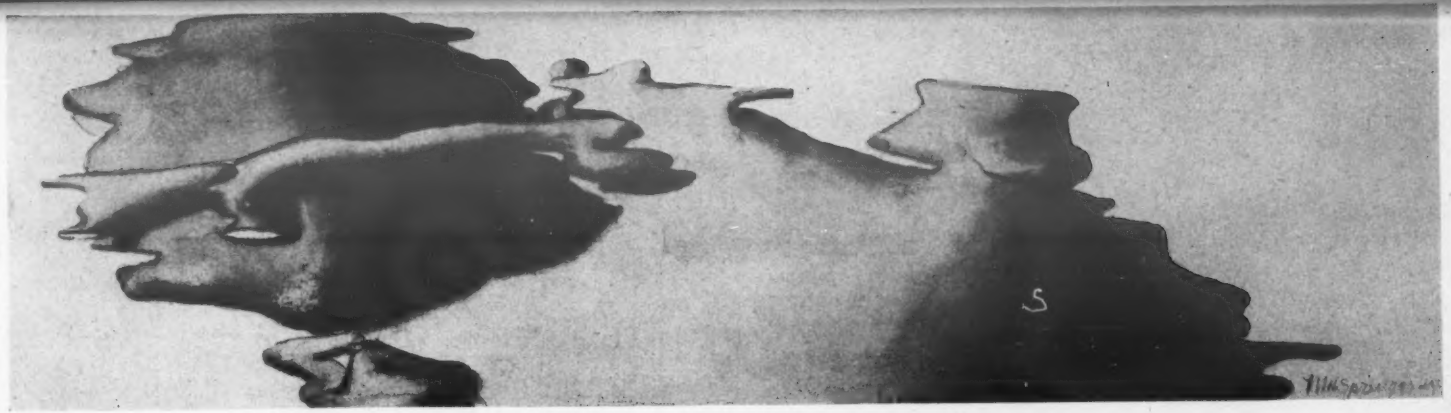


6

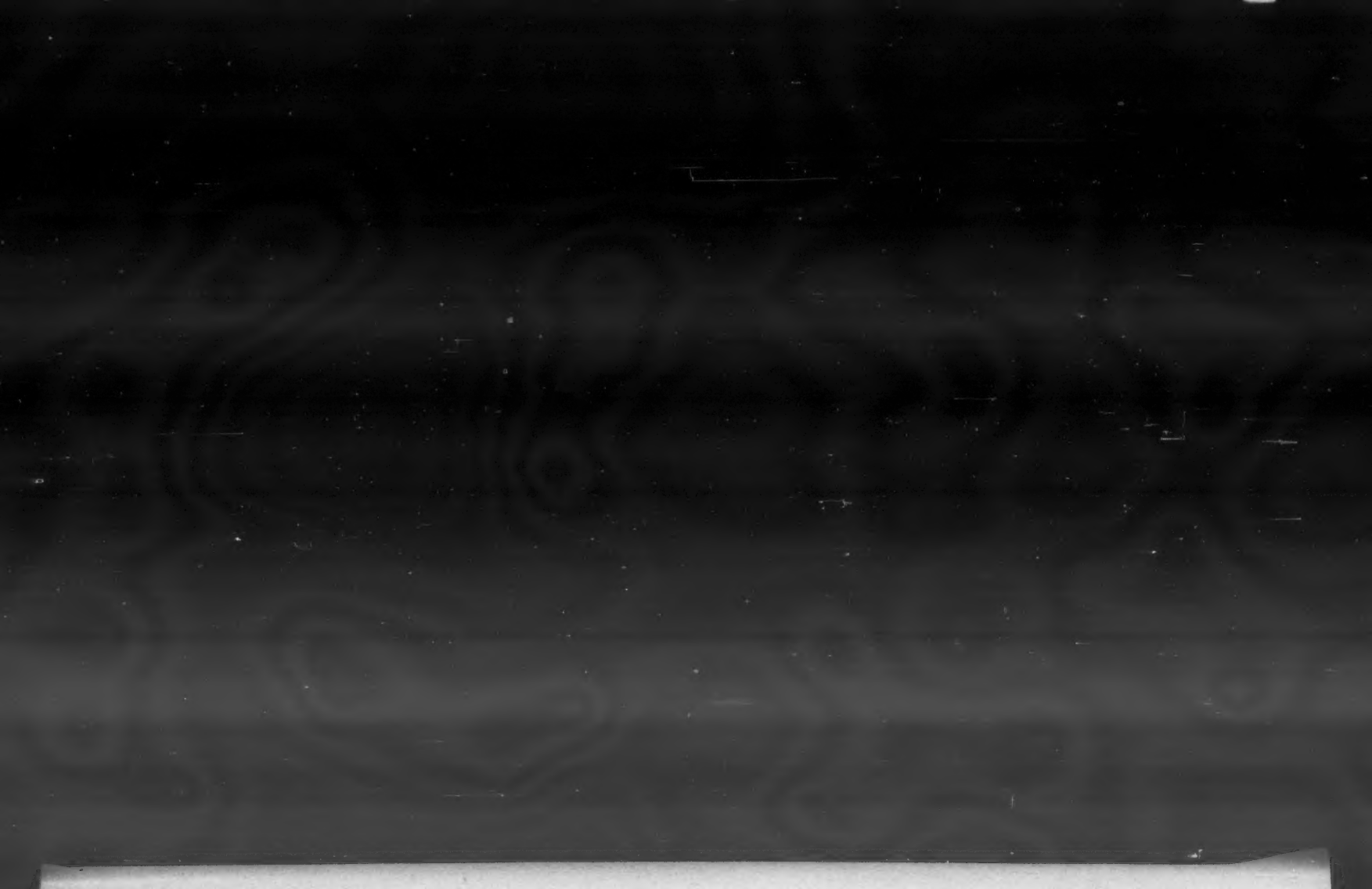
Norris and Tyson

Congenital Polycystic Lung

PLATE 160

FIG. 7. Case 2. Lower lobe of left lung. Photomicrograph shows that a medium-sized bronchus to the left of center is surrounded by atypically spaced cartilages. Mucous glands extend outward between the cartilages. To the right of center are two bronchioles. Narrow openings between the bronchioles and expanded alveoli are apparent. × 45.

FIG. 8. Case 2. Lower lobe of left lung. A high power photomicrograph of a bronchiole seen in Figure 7 shows clearly the communications between the bronchiole and the alveoli and the regularity of the mucosal folds. The interlacing bands of nonstriated muscle between bronchioles are shown also. Some alveoli are devoid of visible epithelium; others are incompletely lined by single layers of cuboidal epithelium. × 185.



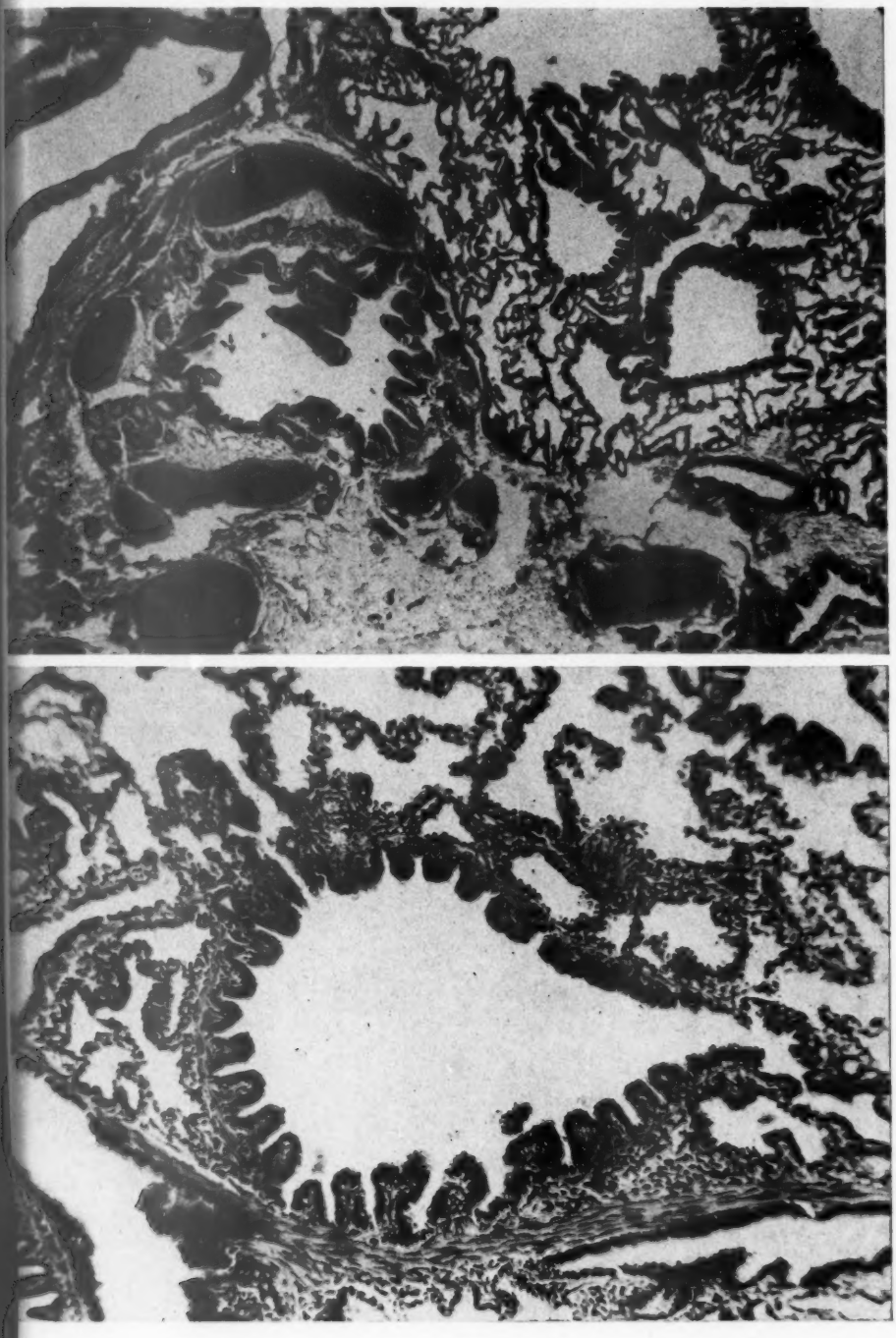


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FIG. 9. Case 2. Lower lobe of left lung. Reconstruction shows the irregularity and cystic dilatations of the small bronchi. Most of the branches are blunt and end abruptly without the usual subdivisions. A few of the branches are slender and undilated. Not shown are the narrow channels of communication between the bronchioles and the alveoli. The approximate vertical extent of the reconstruction in the lung was 1.22 mm.

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Congenital Polycystic Lung

GINGIVAL BIOPSY FOR THE DIAGNOSIS OF GENERALIZED AMYLOIDOSIS*

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Generalized amyloidosis is a frequent complication of many chronic diseases, particularly tuberculosis. Its reported incidence varies with the thoroughness with which it is sought, but in several large series of post-mortem studies it has been shown to occur in approximately 20 per cent of patients with tuberculosis who are autopsied.[1-3] Its clinical incidence is lower than this, probably averaging about 10 per cent of patients with "moderately" to "far advanced" pulmonary tuberculosis. In these patients, the presence of generalized amyloidosis is an important complication in the management of the disease, depriving some of the opportunity for life-saving surgery, and in others being the direct cause of death, especially if massive hepatic, adrenal and, particularly, renal amyloid deposits are present.

For the clinician, the recognition of the onset and the presence of amyloidosis is often difficult, unless the characteristic triad of hepatosplenomegaly, proteinuria, and edema are present. However, this syndrome is usually absent, having been noted, in one careful study, in less than 25 per cent of cases of proved amyloidosis.[4] In the remaining cases, as well as those in which the clinical diagnosis is suspected, the Congo red test is of considerable diagnostic value. Introduced by Bennhold [5] in 1923, this test may detect the presence of amyloidosis even in the absence of clinical findings. It is based upon the "absorption" of the injected colloidal dye by amyloid deposits in the tissues so that upon examination of the circulating blood 1 hour after the injection little or no dye is found remaining. However, this test is deficient in several respects. Frequently, a "positive" test will occur in patients who have no amyloidosis [6] and although definitive criteria can be established to avoid this error,[7] this involves repeated testing, a procedure not altogether innocuous.[8] Secondly, and perhaps more important, there is the inability of the test to demonstrate moderate or small amounts of amyloid; in these cases only limited amounts of Congo red will be removed from the blood stream and much will still be present when a sample is examined 1 hour after injection. But partial absorption is also found in patients without amyloidosis, so that the Congo red test, when it shows incomplete absorption, is without differential diagnostic value.[9] To the present, there is no satisfactory diagnostic method for the determination of amyloidosis in patients with but moderate amounts of amyloid.

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Tissue biopsy for the diagnosis of amyloidosis was used before the introduction of the Congo red test, being employed for this purpose by Josefson [10] (spleen) and Waldenström [11] (liver). Biopsy of the liver has been performed since, but only infrequently since aspiration is not without danger, while laparotomy is not readily acceptable to the patient. Tissue biopsy has also been extensively used in experimental animal studies on amyloidosis, enabling several investigators to fix the time of onset of the amyloid and provide proof of its regression.[12-14] In the uncommon, primary ("atypical") systematized amyloidosis, biopsy has also been done of other tissues, such as skin,[15-17] stomach,[18] muscle,[16,19] tendon,[20] mucous membranes,[21] and tongue.[19,21-23] In these patients, however, gross abnormalities were present and biopsy was done to study the nature of that abnormality: in most cases, the discovery of atypical amyloid disease came as a surprise. The situation in generalized amyloidosis is not comparable. There are no easily available tissues which appear grossly abnormal; certainly, no superficial tissues. The normal appearance of the skin and mucous membranes in generalized amyloidosis gives no indication that they might contain an unusual substance.

Despite this normal appearance, however, there is good reason to suspect that these tissues, particularly the gingivae, might contain amyloid. Amyloid substance, when present, is widely distributed, and there are no grounds on which to expect that it would be absent from the gingivae. Moreover, since perivascular areas are sites of predilection for the deposition of amyloid, the vascular nature of the gingivae makes them a fertile site in which to seek this material. This has been emphasized previously.[1] Technically, biopsy in this region is simple and not forbidding to the patient, can be repeated, and carries no danger. Finally, the anatomic structure of the gingivae is peculiarly suitable to the procedure since the vessels are not only numerous, but are also surrounded by intertwining bundles of fibrous tissue which makes excessive bleeding uncommon. Few pain nerve endings are present [24] and the tissue is also markedly resistant to infection, again making it suitable for the purpose, particularly since it is so accessible.

Material and Method

In an effort to ascertain whether biopsy of oral tissues would be of value in the diagnosis of generalized amyloidosis, a study was recently undertaken at Sea View Hospital in which a number of such examinations were done.

Fifty patients, each suffering from pulmonary or osseous tuberculosis, were chosen for study, but in 3, insufficient material was obtained

for microscopic examination. Therefore, for the purposes of this investigation, the remaining 47 patients will be considered consecutive.

The patients were in three groups. In the first were 18 patients in whom the diagnosis of generalized amyloidosis had been made with considerable confidence. The second group contained 21 patients in whom this diagnosis had been ruled out, so far as was clinically possible. The third group was composed of 8 patients in whom the diagnosis was being considered but could not be made definitely. Every patient was thoroughly studied clinically, complete physical examinations were performed, and laboratory studies done. Urine examinations included multiple tests for proteinuria, casts, red blood cells, specific gravity and, in many instances, protein fractionation for albumin and globulin. Biochemical studies included those for plasma proteins, nonprotein nitrogen, and for hepatic and renal function. Each patient was tested with the Congo red test, in many cases several times. The technic used here was Taran's modification [25] of the Bennhold method, which avoids the inaccuracies caused by colorimetric difficulties in the presence of hemolysis, and which has been accepted as standard by the Committee on Standard Laboratory Procedure of the American Trudeau Society.[26]

The specimens were taken in the Dental Department and consisted of pieces of tissue measuring approximately 3 by 5 mm. Two sites were used, the gingiva proper and the mucobuccal fold, since no information was available as to the suitability of either. Upon sections from each specimen three stains were used: hematoxylin and eosin, Congo red, and methyl violet. It has been the experience of the pathologic laboratories of this institution that methyl violet staining is most satisfactory, and this has been the finding in this study as well. Its only drawback is the difficulty of making permanent preparations. We have found that methyl violet was much more specifically limited to the amyloid material, and gave greater contrast with the non-amyloid tissues, than did Congo red. In the liver, for example, the latter stains the perivascular fibrous tissues a pink-brown shade not unlike the truly pink amyloid; the same difficulty is found in the vicinity of Glisson's capsule. In the gingiva, moreover, the fibrous, hyaline, and muscular elements in the subepithelial layers are also widely stained an off-pink hue by the Congo red, as is coagulated serum, so that differentiation from the amyloid deposits is difficult. When guided by comparison with a section stained with methyl violet, amyloid can be differentiated by Congo red, but when Congo red is used by itself, it is apt to be confusing. With both stains, too, diffuse daylight is preferable. Hematoxylin and eosin we found of little value.

Anatomic correlation was attempted by biopsy of other tissues in several cases, and was obtained at autopsy in others.

Results

The results of our study indicate that biopsy of the oral mucosa, especially the gingiva, can be of considerable value in the diagnosis of generalized amyloidosis.

Our findings in the first group (patients with amyloidosis) are summarized in Table I. Here, in 14 of the 18 patients studied, amyloid deposits were found in the tissues examined. It should be emphasized that these tissues, as in all patients investigated, were grossly normal. These results are more significant when it is realized that the complete picture of hepatosplenomegaly, edema, and proteinuria was present in only 2 of these cases, yet amyloid was demonstrated by biopsy in 13 of the remaining 16. This and other correlations are summarized in Table II. They emphasize that the absence of any one sign or group of signs does not exclude the possibility of finding amyloid by gingival biopsy. In case 14, for example, only proteinuria was present and there was no palpable liver or spleen and no edema. Yet gingival biopsy revealed amyloid. The converse is also true. Our data show that the presence of any one finding or combination of findings is no guarantee that amyloid will be present in a specimen taken for biopsy.

Failure to find amyloid by gingival biopsy does not rule out the disease. In 2 of the 4 cases with no amyloid in the material submitted for biopsy, we had the opportunity of examining other tissues. In both, amyloid was found, leading to the impression that clinical diagnosis, based upon repeated demonstration of complete or nearly complete Congo red absorption,[7] may be correct in the face of negative gingival biopsy. Thus, in case 5, the patient gave every evidence of generalized amyloidosis, including hepatosplenomegaly, edema, marked proteinuria, and repeated complete absorption of injected Congo red, yet careful search of the oral specimen failed to reveal amyloid deposits. One week later, biopsy of the liver showed massive amyloid infiltration. Even more instructive was case 7, which repeatedly showed complete absorption of Congo red, although proteinuria was the only significant finding, edema and enlargement of the liver or spleen being absent. Biopsy in this case, too, was negative. Nevertheless, autopsy 3 weeks later revealed amyloidosis of the liver, spleen, adrenals, and kidneys, with death due to nephropathia amyloidea. It seems obvious that negative biopsy does not rule out generalized amyloidosis. A positive biopsy, on the other hand, is valuable, particularly in circumstances outlined below.

TABLE I
Oral Biopsy in Patients with Clinically Diagnosed Generalized Amyloidosis

Case	Clinical examination: Enlargement: Liver	Clinical examination: Enlargement: Spleen	Clinical examination: Edema	Laboratory studies: Proteinuria	Laboratory studies: Congo red absorption	Laboratory studies: Chemical examination of blood	Clinical diagnosis	Oral biopsy: Site	Oral biopsy: Result	Oral biopsy: Extent	Examination of other tissues
1.	++++*	0	+	++++	100, 100, 100	Hypoproteinemia	Amyloidosis	Mucobuccal fold	Positive	+++	Necropsy: generalized amyloidosis
2.	++++	+	++++	+++	100, 100	Hypoproteinemia	Amyloidosis	Mucobuccal fold	Positive	+	
3.	++++	0	0	++	100, 100, 100	Minimal azotemia	Amyloidosis	Gingiva	Positive	+++	
4.	0	++	0	+	100, 100, 100	Normal	Amyloidosis	Mucobuccal fold	Negative		
5.	++++	+	+++	++++	100, 100	Hypoproteinemia	Amyloidosis	Gingiva	Negative		Liver biopsy: amyloidosis, ++++
6.	++	0	+++	++++	97, 85, 100	Hypoproteinemia, marked azotemia	Amyloidosis	Gingiva	Positive	++	Necropsy: generalized amyloidosis
7.	0	0	0	++++	100, 100, 100	Marked azotemia	Amyloidosis	Mucobuccal fold	Negative		Necropsy: generalized amyloidosis
8.	+++	0	0	++++	100, 100, 100	Minimal azotemia	Amyloidosis	Gingiva	Positive	++	
9.	+++	+	0	++	100, 100, 100	Normal	Amyloidosis	Mucobuccal fold	Positive	+	
10.	++	+++	0	++++	100, 100, 100	Moderate azotemia	Amyloidosis	Mucobuccal fold	Positive	+++	Prostatic resection; specimen, amyloid ++
11.	0	++	0	++	100, 100, 100	Normal	Amyloidosis	Mucobuccal fold	Positive	+	Nephrectomy for renal tuberculosis; specimen, amyloid ++
12.	+++	0	0	++++	100, 100, 100	Minimal hypoproteinemia	Amyloidosis	Mucobuccal fold	Positive	+++	
13.	+	0	0	+++	98, 90, 100, 100	Normal	Amyloidosis	Mucobuccal fold	Positive	+	
14.	0	0	0	++++	100, 100, 100, 95	Azotemia	Amyloidosis	Gingiva	Positive	+	Necropsy: generalized amyloidosis
15.	++	0	0	++++	100, 100	Azotemia	Amyloidosis	Mucobuccal fold	Positive	+	
16.	++++	++++	0	+	100, 100, 100	Normal	Amyloidosis	Gingiva	Positive	+	
17.	+	0	0	+	100, 100	Normal	Amyloidosis	Gingiva	Positive	++	
18.	0	0	0	+	98, 98, 100	Normal	Amyloidosis	Gingiva	Negative		

* Gradings in pluses are arbitrary. A one-plus is definite, albeit minimal; a four-plus indicates massive amounts.

There was no constant correlation between the extent of the amyloid deposits found in the gingivae and the severity of the amyloidosis. In the 2 patients with every sign of amyloidosis, one, as noted, showed no amyloid in the gingival tissue while the other had it in but minimal amounts. There was, in particular, no correlation between the amount in the gingivae and that in the kidney: 4 patients in this group have been autopsied since the biopsy and in the one patient (case 1) who showed rather large amounts in the gingivae, only moderate infiltration of the kidneys was present. The other 3 showed little or no amyloid in the gingivae (cases 6, 7, and 14) yet each had massive amyloidosis of the kidneys. Similarly, in one patient (case 16) in whom the liver and spleen were so large as almost to fill the abdomen, the biopsy specimen contained but a minimal amount of amyloid, found only after a careful search.

TABLE II

Correlations between Findings in Oral Specimens for Biopsy and Absence of Evidence of Visceral Involvement in Generalized Amyloidosis (18 Patients)

	Absent	Oral specimen	
		Positive	Negative
Splenomegaly	11	9	2
Hepatomegaly	5	2	3
Hepatosplenomegaly	13	10	3
Edema	14	11	3
Moderate to marked proteinuria	4	3	1
Complete syndrome of hepatosplenomegaly, proteinuria, and edema	16	13	3

In those cases which comprised group II, we did not have a single positive biopsy. These patients, as mentioned above, had no clinical evidence of amyloidosis, and the Congo red test, in each, had been negative. This experience supports the impression that a negative Congo red test, in the absence of positive clinical findings, usually means that generalized amyloidosis is not present. As will be shown below, however, a negative Congo red test, when other findings are present, has no such meaning.

The results of gingival biopsy in the third group, composed of cases in which amyloidosis was suspected but not proved, are perhaps most interesting of all. They show that biopsy of the gingivae may demonstrate amyloid when all other means fail to establish the diagnosis. The studies in these patients are summarized in Table III, but far more instructive and revealing is an analysis of several of the cases themselves.

TABLE III

Study of Oral Biopsy in Patients with Clinically Suspected but Unproved Generalized Amyloidosis

Case	Clinical examination			Laboratory studies			Clinical diagnosis	Oral biopsy			Examination of other tissues
	Enlargement		Edema	Proteinuria	Congo red absorption	Chemical examination of blood		Site	Result	Extent	
	Liver	Spleen									
40.*	+++	+	+++	++++	61, 57, 93, 59, 64	Hypoproteinemia	Amyloidosis (see text)	Mucobuccal fold	Positive	+++	Liver biopsy: amyloidosis, ++
41.	0	0	++	++++	31, 29, 41, 10	Hypoproteinemia, azotemia	Amyloidosis (see text)	Gingiva	Positive	+	
42.	++	+	+++	++++	40, 23, 50	Azotemia, no hypoproteinemia	Uncertain (see text)	Mucobuccal fold	Positive	++++	Necropsy: generalized amyloidosis
43.	+	0	0	++	31, 50, 59	Moderate azotemia	Glomerulonephritis	Mucobuccal fold	Negative		
44.	+++	+++	0	0	41	Normal	Uncertain; Pott's disease	Gingiva	Negative		Splenic biopsy; no amyloid
45.	++	0	+++	±	68, 50	Normal	Cardiac failure	Gingiva	Negative		Liver biopsy: congestion, no amyloid
46.	+++	0	+++	++++	26, 40	Hypoproteinemia, azotemia	Amyloidosis (see text)	Gingiva	Positive	++++	Liver biopsy: amyloidosis, ++
47.	0	0	++	++++	37, 33	Hypoproteinemia (slight)	Amyloidosis (see text)	Gingiva	Positive	+++	

* Cases 19 to 39 were in group II.

Case 40. J. L. was a white male, 34 years old, whose illness began in 1941. He was found to have bilateral fibrocaseous pulmonary tuberculosis and was admitted to Sea View Hospital in March, 1945. Besides his pulmonary tuberculosis he was found to have a nephrotic syndrome as well, with marked proteinuria, hypoproteinemia, hypo-albuminemia, and extensive edema, most marked in the lower limbs, sacrum, and scrotum but noted in all dependent parts. Since hepatosplenomegaly was present also, a provisional diagnosis of amyloidosis was made. A Congo red test done on August 1, 1945, resulted in 61 per cent absorption, which is not confirmatory of amyloidosis. The test was repeated on November 13, 1945, and 57 per cent absorption occurred. With these results, the diagnosis of amyloidosis could not be made with assurance. Meanwhile proteinuria continued, varying from 4 to 11 gm. per liter and from 7 to 25 gm. in 24 hours. Although the liver decreased in size from 7 to 3 cm. below the costal margin, the edema remained approximately the same and the serum albumin ranged from 1.58 to 2.52 gm. per cent. Fractionation of the urinary proteins showed a considerable amount of globulin, averaging about 30 per cent of the total. Hepatic and renal function tests yielded normal results. Since no other cause for the nephrotic syndrome could be found, it was still felt that renal amyloidosis was responsible. A third Congo red test on April 6, 1946, showed 93 per cent absorption. Since tests in this range are frequently unreliable,[7] biopsy was decided upon.

Biopsy of the mucobuccal fold was performed on May 10, 1946, and revealed fairly large deposits of amyloid, establishing the diagnosis and confirming the clinical impression. Liver biopsy by laparotomy showed amyloidosis in that organ as well.

Case 41. C. L. was a colored male, 31 years of age, who was discovered to have Pott's disease in 1939. A spinal fusion was performed in 1941 and, despite an apparently adequate result, abscesses formed in 1942. These were aspirated, leaving draining sinuses. There were no abnormal findings in this patient's urine for 4 years after the onset of his disease but in October, 1943, rather marked proteinuria appeared and persisted. The liver and spleen were not palpable, but a Congo red test was done to investigate the possibility that renal amyloidosis might underlie the proteinuria. On November 1, 1943, this test showed 70 per cent absorption, a result which is found both in patients with amyloidosis and in those without this complication. Other Congo red tests were "negative," yielding 31 per cent absorption on September 11, 1945, and 29 per cent absorption on March 12, 1946.

Pseudo-arthrosis being present, revision of the spinal fusion was performed on March 18, 1946. Several weeks later the proteinuria doubled in daily quantity, despite oliguria. Increased renal damage was evidenced by the development of moderate azotemia, with urea nitrogen reaching 60 mg. per cent and creatinine, 3.4 mg. per cent. Simultaneously, marked hypoproteinemia was noted, with serum proteins as low as 3.04 gm. per cent and serum albumin 1.33 gm. per cent, with concomitant generalized edema. At the onset of the period of clinical distress a fourth Congo red test was done with 45 per cent absorption. Thus, even though renal amyloidosis was suspected, it could not be proved by the Congo red test. Gingival biopsy was performed on May 25, 1946. Perivascular amyloid was present, and the diagnosis of renal amyloidosis was established. Congo red absorption on September 19, 1946, was 10 per cent.

Case 42. O. D. was a white male, 52 years old, who first showed symptoms of pulmonary tuberculosis in 1941, at which time he was admitted to Sea View Hospital. He was known to have diabetes and to have suffered from recurrent bouts of congestive cardiac failure since 1932. Electrocardiograms showed evidence of arteriosclerotic heart disease. His pulmonary tuberculosis progressed until 1944

but remained quiescent after that. During his hospitalization, he was noticed to have enlargement of the liver, proteinuria, and edema. All these could have been explained by cardiovascular disease, especially since there was no hypoproteinemia or hypo-albuminemia to account for the edema. However, particularly because of the rather severe proteinuria, which was greater than that usually found in renal arteriosclerosis, amyloidosis also was suspected. A Congo red test was done on August 6, 1945, and showed 50 per cent absorption, which is within normal range. During 1945 and 1946 the patient began to show signs of renal failure, with developing azotemia. This again could have been explained by either cardiovascular-renal disease or amyloidosis. Clinically, differential diagnosis was uncertain. On June 4, 1946, a specimen was taken from the mucobuccal fold. Abundant amyloid was found in the biopsy specimen and a diagnosis of generalized amyloidosis was made.

The patient died in uremia on August 15, 1946. Autopsy revealed generalized amyloidosis, with massive involvement of the kidneys, consistent with amyloid uremia.

Case 46. M. S. was a colored female laundry worker, 35 years old, who first developed pulmonary tuberculosis in 1939. Her disease, however, remained quiescent until 1943, when it became reactivated, causing her admission to Sea View Hospital in April, 1944, with caseous-pneumonic tuberculosis. Proteinuria was present on admission and continued during hospitalization. In March, 1945, her liver became palpable. A Congo red test on March 14, 1945, showed 26 per cent absorption and a diagnosis of amyloidosis could not be made. Proteinuria continued and reached 15 gm. in 24 hours with 50 per cent globulin on fractionation of the urinary proteins. In April, 1946, edema appeared, together with hypo-albuminemia. The Congo red test again was negative, showing, on April 9, 1946, only 40 per cent absorption. Gingival biopsy was then performed. Extensive amyloid deposits were present. A liver specimen obtained by laparotomy on May 16, 1946, showed moderate amyloidosis. This provided adequate explanation for the ensuing rise in the nonprotein nitrogen of the blood, the urea nitrogen being 60 mg. per cent on May 22, 1946. The patient died in uremia on June 6, 1946. Necropsy was refused.

Case 47. T. N. was a young Puerto Rican female, who was found to have end-stage caseous-pneumonic tuberculosis in July, 1945. On admission, her urine was normal and remained so until April, 1946, when, suddenly, there was found a 4 plus proteinuria. Although neither the liver nor spleen was palpable, amyloidosis was suspected and a Congo red test was done. It showed 37 per cent absorption on April 23, 1946. This, of course, did not confirm the diagnosis of amyloidosis. The proteinuria continued, averaging 8 gm. in 24 hours, with approximately 40 per cent globulin on fractionation of the protein present (suggestive of renal amyloidosis). In June, 1946, peripheral edema appeared. The Congo red test was again performed on October 2, 1946. It resulted in 33 per cent absorption. The liver and spleen were still not palpable. Gingival biopsy was done on October 23, 1946. Moderately extensive deposits of amyloid were found, and the diagnosis was established.

Comment

It is interesting to note that Congo red absorption, insufficient for a diagnosis of amyloidosis, was found in patients with considerable amyloid in the gingival specimens. It may be that faulty Congo red absorption can be explained by the presence of amyloid of a character

unsuited to chemical or physical affinity with Congo red. We feel, however, that a more likely explanation is simple quantitative insufficiency of the absorbing substance, amyloid. Of the 5 cases of amyloidosis with "negative" Congo red tests, 4 had extensive deposits of amyloid in the gingivae, while of the 14 with positive biopsies in the group with complete absorption, only 4 had heavy deposits. This is further support of the observation that there is no correlation between the amount of amyloid in the viscera and that in the gingiva. This peculiarity of distribution is no doubt responsible for our being able to demonstrate amyloid deposits in the gingivae of patients with what has heretofore been called "moderate amyloidosis." Diagnosis has always been difficult in these patients. In the original report introducing the Congo red test, Bennhold[5] reported a case which showed only 22 per cent absorption and yet was found to have splenic amyloidosis on post-mortem. He offered as an explanation the inability of small amounts of amyloid to absorb much Congo red. This deficiency of the Congo red test has plagued many workers in this field, since approximately 25 per cent of cases of amyloidosis have "negative" Congo red tests, in which but little Congo red is absorbed. Yet small amounts of amyloid does not mean innocuous amyloid, because there may be little amyloid in the body, yet that little may be concentrated in a vital organ, particularly the kidney, and lead to serious consequences. This was clearly the situation in case 46. Therefore, gingival biopsy, when positive, may well have particular value in cases of "moderate" amyloidosis, especially when the Congo red test is uncertain. The irregularity in the distribution of amyloid also limits the value of the gingival examination, as shown by the negative biopsies obtained in 4 of the 18 cases of clinically proved amyloidosis. Whether this is due to the complete absence of amyloid in the gingivae or whether the portion selected for study failed to contain deposits which were present in other areas is not yet known. That the latter explanation is probably the correct one is suggested by our experience that amyloid was often scattered in its distribution, with much of the tissue entirely free of it. In fact, in a number of specimens, it was present in only one limited site, usually perivascular, in a vessel-containing area of the epithelium. In one patient, case 16, we could find no amyloid in the first slide examined. However, in a second section, amyloid was clearly seen about a single blood vessel. Experiences such as this have led us to the conclusion that difficulty with the procedure may be minimized by examining several sections of the biopsy specimen and, in selected cases, taking biopsies from several sites.

Although the Congo red stain was often unsatisfactory, failing to

delineate clearly the amyloid from the fibrous tissue and hyaline structures, we found methyl violet guiltless in this respect, except when metachromatic artificial light or direct sunlight was used. No such difficulties were noticed with diffuse daylight. We did not encounter the irregularities of staining which have made interpretation of biopsy specimens in primary atypical amyloidosis frequently uncertain and which are, in fact, characteristic of that disease.[16,19,21] Similarly, the lack of variation in the methyl violet staining of the amyloid in our cases is consistent with previous experience in generalized amyloidosis, where irregularity of staining is uncommon. Too, in each of the 8 cases of positive gingival biopsy in which we had the opportunity of examining other tissues, the amyloid present therein did not vary in staining qualities from that in the gingiva.

We believe that gingival biopsy may find its principal sphere of usefulness in diagnosis of those cases of amyloidosis in which other methods fail, especially where the Congo red test is negative. In this connection, it should be remarked that mishaps have occurred following the administration of Congo red,[8,27] so that it might be wise, in some cases, to substitute gingival biopsy for repeated Congo red testing. Finally, gingival biopsy may serve one other useful function: to demonstrate, unequivocally, the presence of amyloidosis in cases in which surgical operation or other means of arrest or cure of the primary disease is contemplated, so that the possibility of regression of the amyloidosis might be studied. This use of biopsy has been employed previously by Waldenström[11] who, after subjecting his patients to repeated liver punctures, reported anatomic evidence of the regression of the disease in several cases. However, the use of serial gingival biopsy for this purpose is not without uncertainty, and sanguine expectations as to its value in the study of regression should be tempered.

Summary and Conclusions

1. The clinical diagnosis of generalized amyloidosis is often difficult for the characteristic triad of hepatosplenomegaly, proteinuria, and edema is present in less than 25 per cent of cases of proved amyloidosis. The Congo red test is a valuable diagnostic procedure but suffers from serious limitations: Apparent inability to demonstrate small or moderate amounts of amyloid, occasional false positives, and ill effects consequent upon the intravenous injection of the dye.

2. Biopsy, especially of the liver and spleen, has been employed both clinically and in experimental studies for the anatomic diagnosis of generalized amyloidosis. However, visceral biopsy, although valuable, is limited in its application. Biopsy of the oral tissues, particu-

larly the gingiva, has been investigated to learn whether it would be useful in the diagnosis of amyloidosis. The accessibility of the tissue, its resistance to infection and hemorrhage, and the uncomplicated simplicity and ease of the technic make the procedure inviting.

3. Forty-seven patients were studied. In 18 in whom the clinical diagnosis of amyloidosis had been made with reasonable certainty, amyloid was found in 14, upon gingival biopsy. There was no correlation between the amount in the gingiva and that in the viscera.

4. A negative gingival biopsy does not rule out amyloidosis. It is recommended that multiple sections of the specimen be examined and, if still negative in the face of strong clinical evidence of amyloidosis, that biopsy be repeated in another site.

5. Amyloid deposits were found in the gingivae of patients with but moderate amyloidosis, including those cases in which repeated Congo red tests were "negative." Heretofore, there has been no simple method by which to establish the diagnosis of amyloidosis in these patients.

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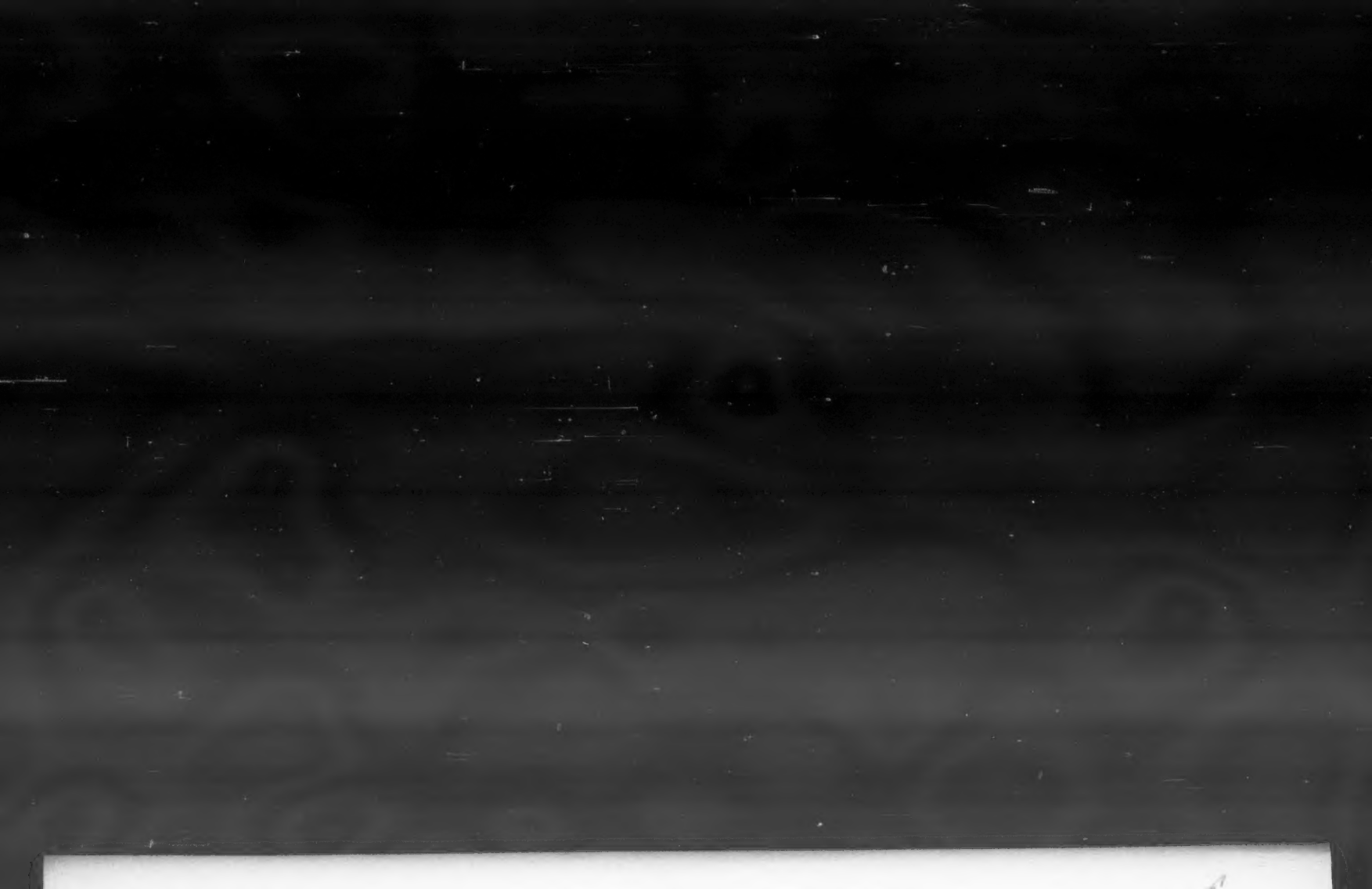
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